

**TOTAL ANTHOCYANIN CONTENT IN BLUE CORN COOKIES  
AS AFFECTED BY INGREDIENTS AND OVEN TYPES**

by

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B.S., Northeast Agriculture University, P.R. China, 1997  
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AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry  
College of Agriculture

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

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## **Abstract**

Anthocyanins, a group of pink to purple water-soluble flavonoids, are well known as naturally occurring pigments credited with numerous potential health benefits. However, they are sensitive to degradation by pH, light, and temperature. Blue corn (maize) is known to be high in anthocyanins (mainly cyanidin 3-glucoside). Citric and lactic acids and glucono-delta-lactone (GDL) are weak organic acids used by the food industry. Reel, convection, and impingement ovens are all used in the baking industry and they use different baking times and temperatures because they have different heat transfer coefficients. Cookies are popular snacks and might serve as a vehicle to deliver antioxidants and fiber. Preliminary tests showed that acids significantly increase the total anthocyanin content (TAC) remaining in the cookies when used at the 1.5% level (flour weight basis, fwb), then plateau up to the 6 % level. The interaction of three acids with three oven types (impingement oven 355F/4min, reel oven 400F/10min, and convection oven 360F/4min) were conducted to investigate their effects on the TAC remaining in blue corn based cookies. Cookie formula was based on AACC method 10-50D. Whole grain blue corn flour to wheat pastry flour ratio (80/20), guar gum level (1%, fwb), and water level (21.5%, fwb) were determined based on RSM analysis. All three acids affected TAC in cookie dough and final cookies by lowering their pH in the dough system. Citric acid retained the most TAC in the cookies. Cookie made with either GDL or citric acid provided larger spread, diameter, area, eccentricity, and crack ratio compared to the lactic acid. All three oven types significantly affected TAC in the cookies. The cookies baked by the convection oven contained the highest level of TAC. Oven types affected cookie spread but not diameter, area, eccentricity, brightness, or crack ratio. Cookies made with citric acid by convection retained maximum TAC ( $227 \pm 3.4$  mg/kg). Cookies made with GDL by convection oven provided the greatest spread, crack, and eccentricity.

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## CHAPTER 1 - Literature Review

### Anthocyanins

Anthocyanins belong to the flavonoid group of phytochemicals, one of the largest classes of phenolic compounds in plants. The flavonoids are defined as substances composed of a common phenylchromanone structure (C6-C3-C6) with one or more hydroxyl substituents.

About 4,000 flavonoids have been identified, many of which are in fruits, vegetables, and cereals. Researchers have reported potentially beneficial effects on human health including antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities (Buhler and Miranda 2000, Prior 2003). A number of studies also show that the dietary intake of flavonoids help in reducing mortality from CHD (coronary heart disease) and prevent stroke (Wrolstad 2001).

### *Chemical structure*

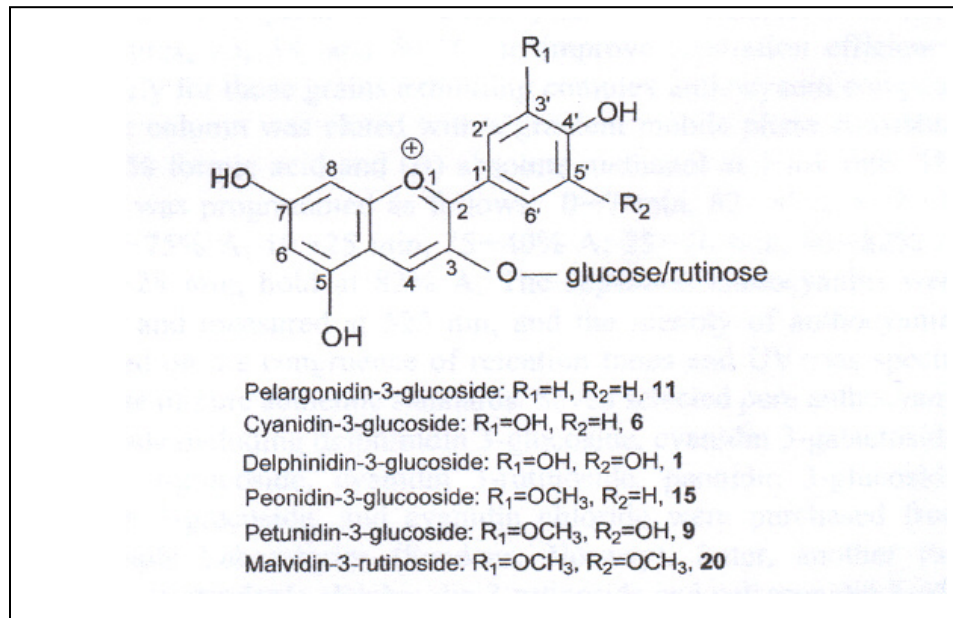
Anthocyanins are responsible for the red, purple, and blue colors of many fruits, cereal grains, and red wines. Additionally, anthocyanins play a major role of plant growth processes as pollination attractants and phytoprotective agents, protecting plants' DNA from damage by sunlight (Sullivan, 1998). Currently, anthocyanins are getting more attentions due to their potential health benefits and status as a natural pigment (Clifford 2000).

The chemical structure of common anthocyanins is as shown in Figure 1. The basic structure is composed of two aromatic rings united by a structure of three carbons. In its native form, if this structure is esterified to one or various sugars, the result is called a simple anthocyanin; if an acyl radical is present in the molecule in addition to the sugar, the result is an acyl anthocyanin (Salinas-Moreno et al 2003).

The anthocyanins are subdivided into the sugar-free anthocyanidin aglycones and the anthocyanin glycosides. Among the known naturally occurring aglycones (the nonsugar component of a glycoside molecule that results from hydrolysis of the molecule) or anthocyanidins, six are most common in nature: pelargonidin, cyanidin,

peonidin, delphinidin, petunidin, and malvidin (Mazza and Miniati 1993). The anthocyanins, anthocyanidins with sugar groups, are mostly 3-glucosides of the anthocyanidins.

Figure 1. Structure of common anthocyanins (Adapted from Abdel – Aal et al. 2006)



### ***Anthocyanins in cereal grains***

A great deal of research has been conducted on the anthocyanins in fruits and vegetables. Anthocyanin levels (mg/100 g fresh weight (FW)) range from 0.25 in pear to 450 in blueberry and more than 700 in black raspberry (Prior 2004). In recent years, increasing attention has been paid to black/blue/purple cereal grains due to their high levels of anthocyanins and their health benefit as dietary antioxidants (Wrolstad 2001). Functional foods (e.g., whole grain products) or functional food colorants (e.g. anthocyanin-rich grain fractions) made from anthocyanin-pigmented or colored grains now draw attention in the marketplace (Abdel-Aal et al 2006). With the popularity of whole grain products, purple wheat is crushed (kibbling process) and commercially used as a topping on multigrain bread (Abdel-Aal and Hucl 1999).

The anthocyanin pigments are present in different locations in various grains. The highest concentration in corn is found in the pericarp (Moreno et al 2005). In blue wheat, the pigments are located numerously in the aleurone layer, whereas they are mostly in the pericarp of purple wheats (Abdel-Aal et al 2006).

Wheat is one of the most commonly consumed human foods in the world. However, blue and purple wheats, which contain relatively high anthocyanin content, are only considered as feed because of their poor baking quality. Breeding programs don't often advance them and only use their color as a marker to distinguish them from those used for human consumption (Zeven 1991). Of the cereals, the highest total anthocyanin content is found in black rice, which is commonly consumed in China as dessert (rice cake or porridge). Bolton (1968) found that cyanidin and delphinidin derivatives in the blue wheat are mainly presents in the aleurone. Dedio et al (1972) claimed that purple wheat contained acylated cyanidin glucoside, acylated peonidin glucoside, and trace amounts of cyanidin rutinoside and peonidin rutinoside, all of which are mainly in the pericarp (Abdel-Aal and Hucl 1999).

Purple corn, originally from Peru, is an Andean crop from low valleys and locally called maiz morado. "Chicha morada", the Andeans' traditional drink made from the purple corn cob, is now recognized as a nutritive powerhouse due to phenolic and anthocyanin content. Purple corn has been identified as a food colorant as well. Numerous studies have focused on the extraction of colorants from purple corn. (Abdel-Aal et al 2006, Gonzalez-Manzano et al 2008, Jing 2006, Jing and Giusti 2007, and Yang et al 2008).

Anthocyanins in other cereals, such as barley, buckwheat, sorghum, and millet, have been described by Mazza and Miniati (1993). Abdel-Aal et al (2006) identified and quantified the anthocyanins in various black, blue, pink, red, and white wheats, barley, corn, rice, and wild rice. The total anthocyanin contents varied in a range of 7-3276 µg/g. The amount in most corn (Shaman blue, cutie blue, cutie pink, purple, and sweet scarlet red, etc) is significantly higher than in the majority of wheats, for example, the red variety (c.v. *Katepwa* / c.v. *Freedom*) and the white variety (c.v. *AC Reed* / c.v. *AC Ron*).

## ***Anthocyanins in blue corn***

### ***Blue corn***

Blue corn or maize (*Zea mays amylacea*.) is an open pollinated flour corn. It has a soft and floury endosperm without dents or wrinkles (Betran et al 2001). It is originally from Peru. Corn has been recorded in many great cultures in the New World, such as the Inca, Maya, and Aztec civilizations. Johnson and Jha (1993) reported on the history of blue corn in both ritual and food uses by the Hopi American Indian tribe and on its modern production practices.

The popularity of floury corns might be because they are relatively easy to reduce into flour, or require reduced time in alkali, both of which are preferred in tortilla and tortilla chip processing (Bertran et al 2001). Blue corn has a number of disadvantages for commercial production. It grows well only in hot and dry regions and is susceptible to diseases and insects. Blue corn varieties have poor stalk strength and often lodge prior to harvest, which causes difficulty with mechanical harvesting. It has low grain yields, which causes the cost of blue corn to be much higher than regular dent corn. Even with these disadvantages, blue corn does well in organic farming application, which fits a unique and growing market. (Betran et al 2001).

### ***Blue corn products***

Tortillas, tortilla chips, baked tortilla chips, posole (a hearty Mexican soup with pork and hominy), atole (a thick, warm drink made with masa and sweetened with cinnamon and brown sugar), and other products are made from blue corn by the process of nixtamalization (Serna-Saldivar et al 1990). The process, also called lime cooking, begins by heating corn in a lime (CaO) solution 1-2 % (5 to 50 min) then steeping (14 hours to overnight) and washing. The resulting product called nixtamal is stone-ground to produce a maize dough (masa), which is further mixed, cut, baked, extruded, or fried into tortilla and tortilla chips. Nixtamalization increases the calcium content, improves niacin bioavailability, removes most of the pericarp, and significantly reduces mycotoxins present in maize kernels (Parra et al 2007). It is also responsible for the formation of flavor and color compounds that impart unique organoleptic characteristics



to the final products (Serna-Saldivar et al 1990, Del Pozo-Insfran et al 2007, Parra et al 2007, Pyler and Gorton 2008, and Walker and Li 2008,)

Tortillas and tortilla chips are popular in Mexico and the USA. The increase in blue corn tortilla consumption in recent years is probably because of more recognition of the potential health benefits of this natural pigment. Additionally, their sweeter flavor and softness, compared to the white or yellow corn tortillas, imparts some additional quality factors (Cortes et al 2006).

There are blue corn products made from corn meal or flour in several cultures: *Chaqueque*, is a thick porridge made from blue corn meal or flour made in the southwestern U.S.; *Atole de maize*, is a creamy thin porridge/drink made from meal, flour or alkaline cooked masa; *Pinole*, a beverage is made from mixed ground, toasted blue corn with sweeteners and other special seeds and ingredients; *Chicos*, is made from dried immature corn kernels after steaming in the husk; and a *Chicha morada*, fermented alcoholic beverage made from soft, purple or deep blue corn (Betran et al 2001).

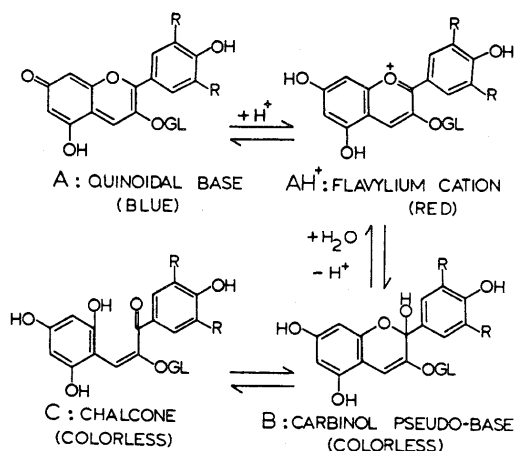
Many new snacks, breakfast foods, and other products are available in health food stores and supermarkets, such as pancakes, muffins, corn flakes, and various extruded snacks. Most of them are made from organic farm grown ingredients and categorized into organic food at a premium price (Betran et al 2001).

### ***Stability of anthocyanins***

Most natural anthocyanins behave differently with pH variation in aqueous phase. They turn “red at low pH, bluish at intermediate pH, and colorless at high pH”. It is believed that four anthocyanin structures exist in equilibrium in acidic or neutral phase. They are the flavylium cation  $AH^+$ , the quinonoidal base A, the carbinol pseudobase B, and the chalcone C (Mazza and Miniati 1993).

At pH below 2, the anthocyanin presents in the form of the yellow ( $R^3 = H$ ) or red ( $R^3 = O\text{-sugar}$ ) flavylium cation  $AH^+$ . When pH increases, it turns to the form of red or blue quinonoidal base A due to the rapid proton loss. On standing, it further switches to the colorless carbinol pseudobase B due to the hydration of the  $AH^+$ , that equilibrates to the open or the colorless chalcone form.

Figure 2. The four structures of anthocyanins which exist in equilibria in aqueous solutions (Adapted from Francis 1989)



The color stability of anthocyanins is influenced by many factors, such as the structure and concentration of the pigment, pH, temperature, light, oxygen, presence of copigments, metallic ions, enzymes, sugars and their degradation products, etc. (Mazza and Miniati 1993).

### ***Structure / concentration***

Brouillard 1982 and other scientists reported that hydroxyl groups, methoxyl groups, sugars, and acylated sugars heavily influence the color intensity and stability of anthocyanins. With the increase of the number of hydroxyl groups on the B-ring, the visible absorption maximum of the anthocyanidin is shifted to longer wavelengths and the pigment changes from orange to blue. The hydroxyl group at C3, C5, and substitution at C4, stabilized the color form by preventing the hydration reaction leading towards the colorless form. At a given pH, the colors of anthocyanin 3-glycosides are more intense than for the 3, 5- and 5-glucosides (Timberlake and Bridle 1975). Increased concentration of anthocyanins in plant tissues, which may vary several folds, enriches their color and may enhance the color stability due to the intermolecular copigment and self-association.

### ***Copigment***

Intermolecular copigments occur when anthocyanins contain two or more aromatic acyl groups. Their color stability is attributed to the organic acids (cinnamic and malonic). Intermolecular copigmentation of anthocyanins with other flavonoids and related substances improves the color intensity and shifts the wavelength of maximum absorbance toward higher wavelengths, which causes the color shift from purple or blue. This is explained by the enhanced hydration reaction between the flavylium cation ( $AH^+$ ) and the colorless carbinol pseudobases (B). The intensity of the copigmentation effect is believed to be influenced by several factors. Increasing the temperature (20 – 80°C) of the medium / solvent significantly reduces the color intensifying effect (Mazza and Brouillard 1990).

### ***Self-association***

“Self-association occurs when the color intensity of the anthocyanin increases more than linearly with an increase in pigment concentration”. It works as a vertical stacking of the anthocyanin quinonoidal bases at pH 7 (Mazza and Miniati 1993).

### ***Research on anthocyanins in blue corn***

Anthocyanins in corn are mainly located primarily in the aleurone layer and pericarp, especially in blue corn the majority is present in the pericarp. Moreno et al (2005) found that cyanidin 3-glucoside, cyanidin 3-(6''-malonylglucoside), and cyanidin 3-(3'', 6''-dimalonylglucoside) are the major (73-87% of the total) anthocyanins in blue corn. Del Pozo-Insfran et al (2006) also concluded that cyanidin 3-glucoside was the major anthocyanin in blue corn, accounting for ~75% of the total anthocyanins. They also reported variation in anthocyanin content due to the genotype. Mexican blue corn contained slightly more anthocyanins (321 mg/kg DW) than did the American blue corn (307 mg/kg DW).

Most research on anthocyanins in blue corn has focused on its most popular products, blue corn tortilla and tortilla chips. More specifically, research focuses on nixtamalization, as that process subjects the maize to pH extremes. The nixtamalization process is very severe, with a pH between 11 and 12, and cooking temperatures above 90°C. Although the time is very short, the process is sufficient to destroy the pigment.

Fossen et al (1998) evaluated the stability of cyanidin 3-glucoside and petanin (a diglucoside anthocyanin with an acyl group) across a pH range of 1 to 9. The results showed that the petanin is stable at a mild alkaline pH (8 – 9) while the cyanidin 3-glucoside is “modified” and less stable when the pH is greater than 5. The possible mechanism is explained by Brouillard (1982): The pyridium ring of the anthocyanin is broken at an alkaline pH. This leads to the blue color of the pigment changing to a pale yellow, and indicates the presence of the ionized chalcon. This intermediate is not stable, so the yellow color disappears at a rate depending on the pH. This is an irreversible reaction. So once this stage is reached, the structure of the anthocyanin can not be regenerated, even in an acid environment, so the pigment has been destroyed.

Salinas et al (2003) claimed that the anthocyanins destroyed by the alkaline cooking process are mainly in the pericarp of maize. Alkalinity also modifies the anthocyanin pattern; specifically, it causes an increase in the proportion of cyanidin 3-glucoside with relation to raw flour. The degradation of acyl type anthocyanins, which are cyanidin 3-(6’’-maloniglucoside) and cyanidin 3- (3’’, 6’’ dimalonylglucoside) might contribute to the increase of cyanidin 3-glucoside. The ester link with the malonyl radical in both acyl type anthocyanins is not stable under the temperature and pH of alkaline cooking process; thus both degrade to cyanidin 3-glucoside (Fossen et al 2001).

Cortes et al (2006) pointed out that the location of the anthocyanins may be another factor. For some maize varieties, the anthocyanins are found only in the pericarp. Most of them are practically degraded during nixtamalization (Salinas et al 2003); For those maize varieties that contain the pigments in both aleurone and pericarp, anthocyanins present in the aleurone remain even after the pericarp anthocyanins are removed by washing. The scientists also studied the effect of different concentrations (0, 0.5, 1.0, and 1.5%) of calcium hydroxide used in nixtamalization after fractionation on the stability of anthocyanins in blue corn. The study showed that both the total anthocyanin content and acyl-type anthocyanin decrease during the nixtamalization after the fractionation process as a function on calcium hydroxide content; whereas the proportion of cyanidin 3-glucoside increases. The loss of total anthocyanins is from 80.3% at 0.5% calcium hydroxide to 85.6% at 1.5% calcium hydroxide.

Del Pozo-Insfran et al (2007) characterized the total phenolics, anthocyanins, and antioxidant capacity contents of blue and white corns of Mexican and American origin during nixtamalization, and subsequent thermal processing into tortillas and chips. An acidified postnixtamalization (fumaric acid, 0.2g/100g, dry corn weight) was evaluated as a means to improve the color and polyphenolic stability. Although similar anthocyanin losses were found for both blue genotype corns ( $\approx 47\%$ ), the American varieties lost more when processed into tortillas and chips than did the Mexican varieties. Acidification after nixtamalization reduced anthocyanin losses for both genotypes, but the protection is more effective on Mexican blue genotype. The occurrence of specific anthocyanin derivatives or the presence of other polyphenolic compounds in this genotype might contribute to the higher anthocyanin stability in Mexican blue corn without acidification (Mazza and Miniati 1993 and Del Pozo-Insfran et al 2007).

Parra et al (2007) reported on the change on total anthocyanin content during the processing into white, yellow, blue and red corn masa, tortillas, and tortilla chips. Raw blue corn contained the highest anthocyanin concentration, whereas the yellow corn had the lowest. The anthocyanin loss in blue corn during a process sequence of lime-cooking, tortilla baking, and tortilla chip frying were 93, 90, and 91%, respectively. This confirmed Salinas et al (2003)'s study, which showed anthocyanin loss from 73 to 100% during nixtamalization. Lime-cooking (nixtamalization) produced the highest negative effect on anthocyanin content due to the synergistic effect of the alkaline pH (around 10) and temperature. Del Pozo-Insfran et al (2006) reported anthocyanin losses in a same sequence of 37, 54, and 75% using different separation and analysis method.

### ***Potential health benefits of anthocyanins***

Various potential health benefits are one of the main reasons that the interest in anthocyanins has recently been raised. A healthy balance between a redox system and free radicals is important to all living organisms. Free radicals, such as nitric oxide, superoxide, and related reactive oxygen species, mediate cells in a signaling process to keep our bodies functional at a normal level (Droge 2002). However, the redox homeostasis could be off balance under stress or extreme environments, generating excessive radicals. The free radicals imbalance accelerates aging and many degenerative

diseases of aging such as cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts, etc. (Ames and Gold 1991, Harman 1992, Ames et al 1993, Davies 1995, Allen and Tresini 2000, Jing 2006, and Jing and Giusti 2007).

Like other flavonoids, anthocyanins and anthocyanidins (the aglycone forms) have antioxidant properties (Wang et al 1997). They work as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists (Larson 1988 and Wang and Lin 2000). The phenolic structure of anthocyanins conveys the antioxidant activity by providing electrons or transferring hydrogen atoms from hydroxyl moieties to free radicals. The common anthocyanidin aglycones are cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin. Cyanidin is the most common anthocyanidin, which is found in 90% of fruits (Macheix et al 1990). Anthocyanin levels (mg/100g fresh weight) range from 0.25 (FW) in the pear to 500 in the blueberry. The cyanidin glucosides likely have higher antioxidant capacity than peonidin or malvidin glucosides, due to the free hydroxyl groups on the 3' and 4' positions of cyanidin (Prior 2003).

Ghiselli et al (1998) extracted three fractions from an Italian red wine which contains single polyphenolic subfractions. Among the three fractions, anthocyanins were the most effective in both scavenging reactive oxygen species and inhibiting lipoprotein oxidation and platelet aggregation. The high anthocyanin concentration in red wine and its antioxidant efficiency might contribute to it. Research on the antioxidant capacity of cherries showed that ORAC (Oxygen Radical Absorbing Capacity) and FRAP (Ferric Reducing Antioxidant Potential) activity are correlated with anthocyanin content. However, the study revealed over a fifty percent loss of anthocyanins in cherries during six months of frozen storage at  $-10^{\circ}\text{C}$  (Wrolstad 2001).

In both *in vitro* and *in vivo* studies, anthocyanins tend to reduce cancer cell proliferation and inhibit tumor formation. This is thought to be related to an inhibition of cyclooxygenase enzymes and potent antioxidant potential. The possible mechanism of anticarcinogenicity at a molecular level: anthocyanins might inhibit tumorigenesis by blocking activation of a mitogen-activated protein kinase pathway. In other studies, fruit extracts with high anthocyanin concentrations proved to be effective against various stages of carcinogenesis, but the individual role of anthocyanins versus other components

was not clear, partially due to its easy degradation (Hou 2003, Kang et al 2003, Koide et al 1997, Meiers et al 2001, Smith et al 2000, and Kandil et al 2000).

Anthocyanins may markedly improve visual acuity. Consumption of black currants or bilberries (blueberry) was reported to improve night vision (Muth et al 2000). It is believed that the enhancement of rhodopsin (a G-protein-coupled receptor localized in the retina of the eye) regeneration by cyaniding 3-rutinoside attributes to this function (Matsumoto et al 2003, Lila 2004).

Research demonstrated the anthocyanins' protection against cardiovascular disease by inhibiting the *in vitro* and *in vivo* oxidation of LDL (low density lipoprotein) by donation of a hydrogen to free radicals with the formation of stable intermediates (Jing 2006). Grape juice or wine also protects from heart attack by reducing inflammation and inhibiting platelet formation (Folts 1998).

McDougall and Stewart (2005), and Lefevre et al (2006) provided evidence of benefits of anthocyanin consumption for diabetes and pancreatic disorders. Multiple and simultaneous biological effects were imputed including preventing generation of free radicals, decreasing lipid peroxidation, reducing pancreatic swelling, and decreasing blood sugar concentrations in urine and blood serum. The roles of anthocyanins in attenuating inflammation, reducing neurological disorder, and improving the immune system, have been documented (Jing 2006, Lila 2004).

### ***Analytical methods***

A lot of research has been reviewed and conducted on qualitative and quantitative determinations of anthocyanin in fruits and cereal grains. The analysis is complicated due to the molecule's ability "to undergo structural transformation and complexation reactions; and they are difficult to measure independently of other flavonoids because they have similar structural and reactivity characteristics" (Mazza and Miniati 1993).

Qualitative analysis generally involves extraction with a weakly acidified alcoholic solvent, followed by concentration under vacuum and separation (centrifuge) of the pigments. One percent hydrochloric acid (HCl) in methanol or ethanol is a common approach to extraction. Paper chromatography on Whatman No. 3 with various solvents is recommended for purification. Identification of anthocyanins has traditionally been

conducted by paper and/or thin layer chromatography (TLC), UV/VIS spectroscopy, and controlled hydrolysis and oxidation (Francis 1982). More recently, high-performance liquid chromatography of anthocyanins, pioneered during the 1970s, has become popular and routine for both preparative and quantitative work (Salinas-Moreno et al 2003, Del Pozo-Insfran et al 2006).

Total anthocyanin content (TAC) is a simple, quick, and widely used spectrophotometric method. Cyanidin 3-glucoside is commonly used as a standard, and absorbance readings have been collected at 535 nm in most blue corn studies since it is the major anthocyanin (Abdel-Aal 1999, Abdel-Aal 2006, Del Pozo-Insfran et al 2006, Parra et al 2007); Chlorinated pelargonidin was used as well (at 520 nm) in some cases (Cortes et al 2006, Salinas-Moreno et al 2003). Abdel-Aal (1999 and 2006) developed a rapid method to quantify total anthocyanins in blue and purple wheat. Absorbance readings were measured at 535nm and calculated (mg/kg) using cyanidin 3-glucoside as a standard.

Del Pozo-Insfran (2007) determined TAC by a pH differential spectrophotometric method. Absorbance readings were collected at two values (pH 1.0 and 4.5) and at two wavelengths (520 and 700 nm) with the standard cyanidin 3-glucoside. The TAC of Mexican and American origin blue corns were determined as 342.2 and 260.9 mg/kg, respectively, whereas the Mexican origin white corn's TAC was not detectable.

## **Whole grains**

In North America, wheat, corn, oat, barley, and rye have been consumed as staple food since European settlement and, in the case of corn or before that. Whole grains, which contain the endosperm, germ, and bran, represented the main portion of the diet in the early years of this country (Salvin 2004). Gristmills were used to for grinding grains at that time so the separation of the bran and germ from the endosperm was not effective and thus led to a low production capacity for refined flour. In 1873, roller mills were introduced and rapidly became widespread due to their higher efficiency in separation and the increased consumer demand for refined products (Slavin 2004).

Whole grains have been believed to be healthy for many centuries. In the 4<sup>th</sup> century BCE Hippocrates, the father of medicine, claimed health benefits of whole grain



bread. In the early 1800s to mid 1900s, physicians and scientists suggested whole grain as a means to prevent constipation. The fiber hypothesis (Trowell 1972), published in the early 1970s, identified that whole foods, such as whole grain, fruits, and vegetables, as the suppliers of fiber and other health-beneficial components.

In the whole grain kernel, the endosperm is the main component (about 80% of the whole kernel), whereas the germ and bran vary in proportion depending upon the cereal. The endosperm supplies energy for the seed growth. Starch is the main component of endosperm, which accounts for about 50-75%. The endosperm contains about 8-18% storage protein, along with cell-wall polymers, and relatively small amount of vitamins, minerals, fiber, and phytochemicals.

The germ consists of the plant embryo and scutellum. It is rich in protein and lipids, but is a low percent of the dry weight of the kernel (typically 4-5% in wheat and barley). External to the endosperm and germ is the bran, a composite structure which protects the grain from the weather, insects, moulds, and bacteria. Bran (including the aleurone) and germ hold a majority of the kernel's nutrients, including high concentrations of B vitamins (thiamin, niacin, riboflavin, and pantothenic acid) and minerals (Ca, Mg, K, P, Na, and Fe), and certain basic amino acids (arginine and lysine). The American Association of Cereal Chemists International (1999) defined a whole-grain ingredient as "... the intact, ground, cracked or flaked caryopsis, whose principal anatomical components, the starchy endosperm, germ and bran, are present in substantially the same relative proportions as they exist in the intact caryopsis". That means in order to qualify as "whole-grain", the three major components of any ingredient, bran, germ, and endosperm, must be hold in the same amounts that were present in the grain's native state. The health claim standard for whole-grain food in the USA requires that the food must include 51% wholegrain flour by weight of final product and must contain 1.7g dietary fiber per serving (Slavin 2004).

Among the numerous phytochemicals, some common in many plant foods (phytates and phenolic compounds) and some unique to grain products (avenanthramides, avenalumic acid) which contribute to its high antioxidant activity. In the modern flour milling process, bran and germ are removed in order to increase the baking quality of flour. Because of this, consumers have to sacrifice the partial loss of nutrients (Miller et

al 2002). Lamsal and Faubion (2009) reviewed the health benefits of whole grain cereals, especially wheat bran, combined with probiotic and dietary fiber components.

### ***Whole grains and dietary fiber***

“Dietary fiber is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, blood cholesterol attenuation, and/or blood glucose attenuation.” (AACC 2001)

The definition of dietary fiber has gone through a process of evolution. Since 1975, most scientists have adapted the AOAC International Official Methods of Analysis and the AACC Approved Methods of Analysis for dietary fiber research. They have been used for routine analysis since 1981 (AACC Dietary Fiber Technical Committee 2003). During the 1980s, dietary fiber was subcategorized into soluble fiber and insoluble fiber. Both types are present in various amounts in plant foods and are “not digestible by appropriately chosen enzymes” (Prosky and DeVries 1992).

Soluble fiber is soluble in warm or hot water but is precipitated when that water is mixed with four parts of ethyl alcohol. Soluble fiber consists of gums,  $\beta$ -glucans, and pectins in oats, barley, and fruits, especially bananas, grapes, apricots, and cherries. Insoluble fiber is not soluble in hot water and is usually present as cellulose, hemicellulose, lignin, cutin, and plant waxes, etc. The source of insoluble fiber is vegetables, wheat bran, and etc (Lam 2000).

Both soluble and insoluble fibers provide bulk in the large intestine and encourage bowel regularity. However, each has distinct chemical characteristics and physiological effects. The function of soluble fiber is to absorb water in the intestinal tract and slow down the amount of time needed to empty the intestine. Eating soluble fiber increases satiety and that may help in weight loss. Soluble fibers also help to lower LDL cholesterol levels in the blood. Insoluble fiber draws water into the intestinal tract, but rather than slowing down digestion, actually speeds it up and increases the amount and frequency of bowel movements (Lam 2000).

The Food Nutrition Board (FNB) of the Institute of Medicine of the National Academies published definitions for “dietary”, “functional,” and “total” fiber in 2002 (AACC Dietary Fiber Technical Committee 2003). However, many scientists believe these definitions may lead to consumer confusions because all fiber is “functional” and it is not possible to analytically differentiate between a compound that is intrinsic fiber or artificially supplemented “functional fiber” with the current technique (Bruinsma 2007). Although there are many sources of dietary fiber, some of them are more effective. They will be discussed in more details below.

### ***Hydrocolloids/gums***

Hydrocolloids are defined as “a range of polysaccharides and proteins that are nowadays widely used in a variety of industrial sectors to perform a number of functions including thickening and gelling aqueous solutions, stabilizing foams, emulsions and dispersions, inhibiting ice and sugar crystal formation and the controlled release of flavors, etc.” (Williams and Phillips 2000). Hydrocolloid use by the food industry has increased dramatically in recent years. They are functional, but have large effects on the textural and organoleptic properties of food products.

Gums are long-chain, high molecular weight, polysaccharides (complex carbohydrates), usually still containing traces of protein. By definition, they are fat-free. Most of them are naturally occurring and water soluble. At low concentration, they work as thickening agents, gelling agents, water-binders, foam stabilizers, lubricants, and fat replacer. Some gums stabilize emulsions, enhance encapsulation, and inhibit crystallization (Ward and Andon 1993, Van Nieuwenhuyzen et al 2006)

### ***Guar***

Guar gum is obtained from guar seeds. It is a polymer of the sugars mannose and galactose at a ratio of 2:1, and 3-6% protein. Like other gums, guar gum has a high dietary fiber content (80-85%). It possesses the highest hydrating capacity of all natural gums. Guar gum swells in cold water and the solution is stable over a pH of 4.0 – 10.5 with good thermostability (Pyler 1988, Ward and Andon 1993).

Guar solutions are non-Newtonian and pseudoplastic in nature. Consequently, viscosity decreases greatly when shear rate increases. Viscosity, hydration rate, and

dispersion properties may vary depending on the conditions under which the gum was processed.

The principle food functions of guar gum included moisture retention, shelf-life extender, stabilizer, thickener, and whipping property improver (Ward and Andon 1993). It also improves the volume and softens the texture of the baked goods. Guar gum is widely used in dry cake mixes, baked goods, rolls and bread, and icings. The recommended usage level is 0.1 to 0.3%.

Guar gum's contributions to gastrointestinal function and lipid and carbohydrate metabolism are well acknowledged (Ellis et al. 2001). Jenkins et al (1976, 1977) tried bread supplemented with guar as breakfast for healthy and diabetic subjects. They found a contribution of guar gum in attenuating the postprandial rise in blood glucose and insulin concentrations. This result has been confirmed by many research groups (Morgan et al. 1979, 1990; Ellis et al. 1981, 1991; Fairchild et al. 1996). Ellis et al (2001) claimed that "guar gum reduces the rate and/or extent of digestion and absorption of available carbohydrate in the gastrointestinal tract".

Clinical tests have shown that guar gum has a therapeutic activity in the treatment of diabetes, hyperlipidemia (total and LDL cholesterol-lowering properties), and obesity (by improving glycemic control, insulin sensitivity, and lipid metabolism; increasing feelings of satiety and satiation and reducing feeling of hunger and appetite) (Ellis et al. 2001).

Consumption of guar gum was found to decrease peripheral blood insulin concentrations. This was explained by suggesting that guar gum helps to either decrease insulin secretion (Ellis et al. 1995) or increase insulin hepatic extraction (Fairchild et al. 1996), or both. The mechanism is still not clear due to the lack of knowledge of physicochemical properties of digesta, an extremely complex heterogeneous material. However, it is well accepted that guar gum improves insulin sensitivity.

### ***Agar***

Agar is extracted from various species of seaweeds or algae that belong to the *Gelidium*, *Gracillaria*, *Eucheuma*, and *Furcellaria* genera. Chemically, it consists of a polymer of galactose and galactose sulfate. It is insoluble in cold water, slightly soluble in warm water, and readily soluble in boiling water. Agar can absorb about 100 times its

weight of water. It forms a strong gel upon boiling and subsequent cooling (Van Nieuwenhuyzen et al. 2006). Agar is unique in that its gelation temperature is far below the gel-melting temperature. For example, the 1.5% agar solution gels in the range of 32-39°C (89.6 – 102.2°F) but its melting temperature is 85°C (185°F) (Ward and Andon 1993, Pyler 1988).

Because of these characteristics, agar can be added to stabilize icings or glazes by retarding water migration, to prevent adhesion of the sugar coating to the wrapper, or used as a stabilizer in pie fillings, meringues, cookies, and similar products (Ward and Andon 1993).

### ***Carrageenan***

Carrageenan, is extracted from Irish moss, a red seaweed species. It is a water soluble gum. Chemically, it is a sulfated linear polysaccharide of D- galactose and 3, 6-anhydro-D-galactose. Its sulfate groups with charged amino acids in proteins to form stable gels or act as thickeners. Because of this, it is added to icings that contain eggs and milk as stabilizer (Ward and Andon 1993).

Carrageenan consists of three types of polymers; kappa, iota, and lambda, which vary in degree and location of the sulfated ester groups and the linkage of the repeating units. Kappa carrageenan is usually used in breadings and batter mixes to strengthen and extend the protein ingredient in the mix, in pasta products to improve the freeze-thaw stability, in piping gels, and bakery jellies to form rigid gels with locust bean gum, and in frozen dough products to prevent ice crystal formation. Lambda carrageenan is a non-gelling polymer. It improves moisture retention and contributes viscosity to sweet dough products. Iota carrageenan is used in many fruit applications due to its propensity to its propensity to gel in the presence of calcium ions (Pyler 1988).

### ***Gum Arabic***

Gum Arabic, or acacia gum, is an exudate of the tropical acacia tree. It is a heteropolysaccharide consisting of an arabinogalactan complex (about 88%), an arabinogalactanprotein complex (10.4%) and a glycoprotein fraction (about 1.2%).

Gum Arabic is one of the most soluble of the gums. Its concentration in solution can be as high as 50% (w/v), which indicates it is not an effective gelling agent but

provides a smooth and glossy surface on some flat icings and glazes. Gum Arabic is commonly used in the food industry for its “emulsifying properties, low viscosity, high fiber content, water binding capacity and adhesive and film-forming properties” (Ward and Andon 1993). It is considered a texturizer and bulking agent in powdered bakery mixes due to high dietary fiber and non-caloric properties (Anderson and Andon 1988).

### ***Cellulose gum and HPMC***

Cellulose gum, sodium carboxymethylcellulose, also known as CMC, is obtained by the chemical modification of cellulose. It includes both carboxymethylcellulose and methylcellulose. Cellulose gum improves bread shelf life and holds moisture in cookies and cakes. It also retards ice crystal growth in frozen dough and improves its freeze-thaw stability. Methyl cellulose is another derivative of cellulose. It has been used to increase the moisture content of donuts and microwavable cakes (Ward and Andon 1993).

### ***Locust Bean Gum***

Locust bean gum, or carob gum, is obtained from the locust or carob bean’s seeds. Chemically, it is a polymer consisting of mannose and galactose sugar units at a ratio of 4:1 (Ward and Andon 1993).

It is slightly soluble in cold water, but swells rapidly in hot water (80 C / 176F) to form a viscous mucilaginous solution (Pyler 1988). It acts as a water binder in bread dough at 0.15% and prevents water from boiling out in fruit pie fillings at 0.1-0.2% (Ward and Andon 1993).

### ***Xanthan***

Xanthan gum is a fermentation product of the microorganism *Xanthomonas campestris*’ fermentation (Pyler 1988). It is a polymer of repeating units of D-glucose, D-mannose, and D-glucuronic acid and has been approved for use in foods in many countries including the U.S. and Canada. Xanthan gum possesses the advantage of being extremely pseudoplastic. This property is important when pumping gum containing liquids. It acts as water binder, preventing water migration to the pastry from the fillings. It is also believed to retard starch retrogradation and can be used as a shelf-life extender

(Ward and Andon 1993). Compared to guar gum, it is more heat stable and has better acid and shear stability. It works well in thermally-processed and microwavable foods.

### ***Whole grains and health***

There is abundant evidence to suggest the health benefits of eating whole foods including grains, fruits, and vegetables, but there is less direct link between these health benefits and eating individual nutrients or phytochemicals. Some of the health benefits associated with a high-fiber may be credited to other components, not just from fiber itself. For example, whole-grain foods contain various phytochemicals, such as phytoestrogens, antioxidants, and phenols, which together with vitamins (E) and minerals (Selenium), may play a vital role in disease prevention (Slavin 2001).

### ***Whole grains and cardiovascular disease (CVD)***

CVD is the leading cause of death and disability of both men and women in The USA (Jacobs et al 2000, Anderson 2002). There is strong epidemiological and clinical evidence relating the consumption of whole grains to a reduced risk for coronary heart disease (CHD).

In Morris et al's (1977) study, the intake of soluble fiber, such as pectin and guar, was related to the lower rates of CHD. Soluble fiber accounted for small, but "significant decreases in total cholesterol". The plant sterols, such as beta-sitosterol may lower cholesterol as well. Other compounds in whole grains, such as antioxidants, phytic acid, lectins, phenolic compounds, amylase inhibitors, and saponins have been shown to "alter risk factors for CHD" (Cook and Sellin 1998). Additionally, whole grains are rich in dietary fiber, resistant starch, and oligosaccharides. Those indigestible carbohydrates reach the colon and are fermented by intestinal microflora into short chain fatty acids (SCFA) that are related to lowered serum cholesterol and a decreased risk of cancer (Cook and Sellin 1998). It is probably a "whole package", rather than any single component that accounts for its protective effects in CHD.

### ***Whole grains and cancer***

A lot of research reveals that the consumption of whole grains reduces the risk of colorectal cancers, polyps, pancreatic cancer, mouth, throat, upper digestive tract, and

endometrial cancers (Kasum et al. 2001 and 2002, Salvin 2004). Epidemiological research shows that higher serum levels are related to an increased risk of colon, breast, and possibly other cancers. The reduction of insulin levels by whole grains consumption might be an indirect way to reduce the possibility of cancer.

Several theories have been forwarded to explain the protective effect of whole grain against cancer, but according to Salvin (2004), none have obtained consensus agreement. Further, there is not sufficient evidence to prove that individual dietary factors, such as fiber, vitamin B6, and phytoestrogen intake, or lifestyle factors such as exercise, smoking, and alcohol use, which are controlled for in most epidemiologic studies, reduce cancer occurrence. Again, it might be the “whole-grain package” that is effective.

Dietary fiber is a component of whole-grain package as well. Increased fecal bulk and decreased transit time limit the interaction between fecal mutagens and the intestinal epithelium. Secondary bile acids are considered to favor cell proliferation, thus enhance the occurrence of mutation and abnormal cell multiplications. Dietary fiber might play an important role in binding or diluting of bile acids (Salvin et al 2001).

Whole grains are a source of selenium (Se), although Se content depends on soil Se content. The metal is thought to be a cofactor for glutathione peroxidase, an enzyme that protects against oxidative tissue damage. Se suppressed cancer cell proliferation at high levels in *in vitro* lung, prostate, colorectal, and skin cancer studies (Clark et al. 1996). Vitamin E (present in the germ) has been described as a cancer suppresser. Lignans are protectors in hormonally active compound- mediated diseases. More speculatively some antinutrients in whole grains, such as protease inhibitors, that may help inhibit the formation of carcinogens and block the interaction of carcinogens with cells (Manson et al. 2000).

### ***Whole grains and blood glucose***

Epidemiological studies consistently show a clear inverse relation between the consumption of whole grains and the risk of type 2 DM (diabetes mellitus) (Van Dam et al. 2002, Murtaugh et al 2003). Whole grains are now officially recommended by the American Diabetes Association for DM prevention. Whole grains affect glucose and insulin responses, partly due to their slow digestibility. Glycemic index (GI) measures the



blood glucose response to a standard amount of a specific food. Foods with low glycemic indices produce a lower rise in blood sugar and blood insulin, which is the reason why the cereal-fiber style is preferred for diabetics (Meyer et al. 2000, Liu et al. 2000). The prevention of hyperinsulinaemia by whole grains' dietary fiber, Mg, and vitamin E might contribute to insulin metabolism. Additionally, whole grains may also influence the insulin level through body- weight regulation and satiety enhancement.

### **Acidulants in food**

Humans perceive five primary flavors: sweet, sour (acidic), salty, bitter and umami. Consequently one reason a food may contain an acid in the formula is to contribute characteristic taste. Citric acids, lactic acid, and glucono-delta-lactone (GDL) are acidulants commonly used in specific food applications. All are under the category of organic acids. Their degree or intensity of sourness varies in the decreasing order of citric > lactic > GDL at equal concentration (Watine 1995).

“The sour taste response imparted to a food is attributed to the hydrogen ( $H^+$ ) or hydronium ( $H_3O^+$ ) ions”. However, sourness is believed to be independently affected by “concentration, pH, and the anion species of the acid” (Berry 2001). The effect of free anions associated with different acids might be responsible for these differences.

In addition to contributing tartness or sourness to the products, the functions of food acidulants include: pH adjustment, preservation through pH reduction, flavor modification and enhancement, sweetness modification of sugars and other sweeteners, that is by pH charge control of gelation and maintenance of viscosity in confections and gelatine desserts, and texture development in dairy products (Butters 1986, Dziezak 1990).

#### ***Citric acid***

Citric acid, 2-hydroxy -1,2,3- propanetricarboxylic acid, is a tribasic acid. The molecular formula is  $C_6H_8O_7$  ( $CH_2COOH-COHCOOH-CH_2COOH$ ).

Citric acid is a common metabolite of plants and animals and is naturally present in both. The amount of citric acids in fruits is relatively high, ranging from 4-8% in lemons and limes to 0.6-0.8% in strawberries. It is a colorless, odorless, crystalline solid with a strong acidic (tart) taste. It is highly soluble in water and alcohol. In food, it usually functions as an acidulant, flavor, modifier or enhancer, or dispersing agent (Berry 2001). It is commonly added, along with sodium bicarbonate, to enhance leavening (Amrein et al 2004). The recommended usage level of citric acid varies depending on the alcohol application. It is reported up to 1200 ppm in baked goods, 1600 ppm in ice cream, 25,000 ppm (dwb) in processed cereal-based foods for infants and children, and 40, 000 ppm in processed cheese (Berry 2001).

Adams et al (2002) studied the effects of amount and type of leavening compounds on the properties of flour tortillas. Compounds tested included citric acid, monocalcium phosphate (MCP), sodium aluminum sulfate (SAS), SALP, GDL, sodium acid pyrophosphate (SAPP-28), and the leavening base sodium bicarbonate at low, medium, and high levels. The study showed that tortilla pH was not affected by the amount but by the type of leavening system. Tortilla diameter was not affected by amount or type of acid except that the tortillas with high levels of MCP were smaller than those with medium SAS and high SALP levels. Addition of citric acid was reported to provide a stronger protein network, thus increased rupture force and longer shelf stability. Thus, selection of acid can affect important functional properties.

In Porres et al's (2001) study, supplementation by citric acid and other treatments were tested for their ability to break down phytate in whole wheat bread. The addition of citric acid enhanced phytate degradation from 42% in the untreated bread to 69% in the final breads. This is desirable for nutritional enhancement. The combination of microbial phytase and citric acid further increased phytate reduction. Compared with the untreated bread flour, citric acid alone, and combination with phytase enhanced total iron dialyzability by 12- and 15- fold, respectively, whereas the treatment of phytase, citric acid, and ascorbic acid improved total iron dialyzable by 24-fold.

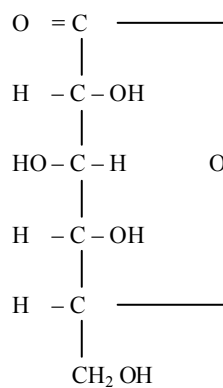
The application of citric acid was found in biscuits (Chevallier et al 2000), cookie and cracker doughs (Levine and Smith 2005, Gokmen et al 2007) with other chemical leaveners including ammonium bicarbonate, sodium bicarbonate, and /or sodium acid

pyrophosphate (SAPP). In Zisu and Shah's (2007) study, by pre-acidification of milk citric acid was reported to reduce mozzarella cheese's hardness and increase its meltability.

### ***Glucono-delta-lactone (GDL)***

Glucono-delta-lactone (GDL), is the cyclic 1, 5- intermolecular ester of D-gluconic acid. The molecular formula is  $C_6H_{10}O_6$ . The structural formula is shown in Figure 2.

Figure 3. Structural formula of GDL



GDL is found naturally in honey, grapes, and other fruits. It is an odorless, white crystalline powder with a neutral taste. It is soluble in water. In cold water, it hydrolyses slowly to an equilibrium mixture of gluconic acid, its delta and gamma lactones, and produces acidic taste. GDL is used as an acidifier and flavor enhancer. Additionally, it is found to enhance the preservative action of preservatives like benzoic acid, sorbic acid. Because of its low, nearly neutral flavor, GDL can be added at levels producing lower pH values and thus reducing required preservative levels. GDL is unique in that it develops its acidity slowly and produces a mild taste. It is often used in dessert mixes, bakery mixes, salad dressings, seasonings, cured meats, sausages, processed cheese, and fish products (Berry 2001, Watine 1995).

### ***Lactic acid***

Lactic acid, 2-hydroxypropanoic acid, also known as milk acid, is a monocarboxylic acid. The molecular formula is  $C_3H_6O_3$  ( $\text{COOH-CHOH-CH}_3$ ).

Lactic acid is widely found both naturally and as a product of *in situ* microbial fermentation, such as that in sourdough bread, yoghurt, buttermilk, cheese, and other fermented foods. It exists three isomers, L- (+), D-(-), and DL forms. Pure, anhydrous, lactic acid is a white crystalline solid and is highly miscible with water and alcohol. The aqueous solution is colorless or yellowish, hygroscopic syrup, liquid (Berry 2001).

Lactic acid has a mild acid taste compared to that of most other food acids. It doesn't mask or overpower the weaker aromatic flavors of foods. It is used as an acidifier, pH adjuster, flavor enhancer, and inhibitor of microbes in bakery, confectionary, dairy, and meat products.

In sourdough bread, lactic acid is one of the main compounds provided from lactic acid bacteria and yeast (less) fermentation. The ratio of lactic acid to acetic acid is important for sour dough bread's final flavor (Salim ur et al 2006, Corsetti and Settanni 2007).

Lactic acid is reported to be used for measuring the bread making properties of hard winter wheat flour (Xiao et al 2006). It is more commonly used in the solvent retention capacity (SRC) method to predict the quality of soft wheats for commercial baking. The lactic acid (5%) SRC test shows a positive correlation with glutenin content (Gaines 2000 and 2004, Colombo et al 2008)

Lactic acid is used for a replacer of cream of tartar in hard-type biscuits and cream crackers at 0.45 and 0.3% (fb) respectively. It is reported to provide higher specific volume and reduced sponge fermentation time in bread system. Applications in rye or rye-wheat breads have also been reported (Dziezak 1990, Kuipers 1992).

## **Baking ovens**

Since temperature is one of key factors to affect anthocyanins' stability, the use of different types of ovens were considered due to the different heat transfer coefficient.

Conduction, convection, and radiation are the three basic mechanisms of heat transfer. One or any combination of the three is/are involved in baking. Conduction is the result of direct contact between hotter and cooler molecules. The formula for heat transferred by conduction is:

$$Q/t A = k(T_H - T_C)/d$$

where Q is the energy transmitted per unit time, t, and unit area of contact between the two surfaces, A. The constant k is the thermal conductivity coefficient in Btu/hr/ft<sup>2</sup>/F° for thermal conductivity coefficient. It is a function of the type of material. Metals have a relatively large coefficient but insulators have a relatively small one. T<sub>H</sub> is the temperature of hot object and T<sub>C</sub> is the temperature of the cold objective. d is the distance which heat must be transferred.

The formula for heat transferred by convection is similar:

$$Q/t A = h (T_H - T_C)$$

where Q, t, A, and T have same definitions as for conduction, but there is no depth or thickness term. The constant, h, in Btu/hr/ft<sup>2</sup>/F° is the convective heat transfer coefficient. Ovens may have an h of 2 for natural convection up to 20 for forced convection, and as high as 40 for some impinged-air designs.

Any object can transmit energy by radiation to a cooler object, but in practice the source needs to be several hundred degrees hotter than the object to be heated to transmit a significant amount of energy. Radiation works well for raising surface temperature, but not well for penetrating far beneath the surface, where the transfer rate is related to internal thermal conductivity. The formula for heat transfer by thermal radiation is:

$$Q/t A = \epsilon s (T_H^4 - T_C^4)$$

Where e is the emissivity constant, which ranges from 0 for a brightly polished, highly reflective surface to 1 for a perfect absorber, a so called “black body”. s is the Steffan-Boltzman constant, one of the fundamental constants in nature. The temperature terms here are absolute temperatures (Walker 1997).

During baking, a dough is “normally surrounded by a relatively moist, cool, stagnant boundary layer near its surface” (Walker 1992), where radiant heat can easily pass through but not natural convection currents. Impingement ovens can direct the air to the surface rather than across it, so that the thickness of the boundary layer is reduced, thus greatly improving the heat transfer efficiency.

The data for several foods baked in impingement ovens shows significant quality difference over those baked in convectional ovens. The driving temperature (air

temperature minus product temperature) multiplied by baking time is substantially reduced to about 50% of that required in convectional natural convection ovens. The same study also showed that the impingement ovens do not dry most products as much as does convectional baking. Although the moisture loss rate,  $\text{g/cm}^2/\text{min}$ , is higher than in a convectional oven, the baking time is substantially shorter, and therefore the net moisture loss is less with the high velocity impingement ovens than with convectional ovens. This holds the potential for improved texture and shelf life (Walker 1992).

Natural convection oven (reel oven), convection, and impingement ovens are commonly used in the baking industry (Figure 3). The different baking principles employed by each type oven indicate different optimum baking times and temperatures in their applications.

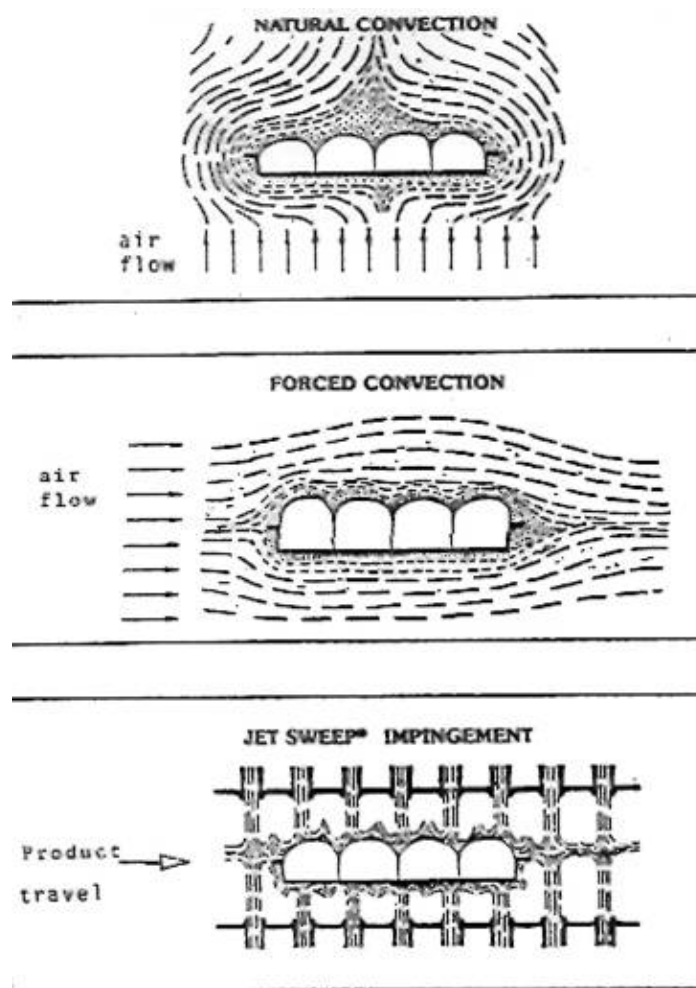
The reel oven has “a reel structure that revolves vertically around a horizontal axis within the baking chamber and supports the baking trays in Ferris-wheel fashion”. It is normally heated by direct firing, either electricity or gas, heating elements being located centrally across the floor of the baking chamber. A baffle above the gas burners converts part of the convection heat into radiant heat and help maintain balance of both in the oven. This allows the heat to circulate around the reels and to the top of the oven chamber, which minimizes location of differences in temperature (Pyler 1988, Eapen1991).

Convection is the transfer of heat from one part to the other within a gas or liquid by the gross physical mixing of one part of the fluid with another (Matz and Matz 1978). A convection oven uses forced air to circulate heat evenly throughout the cooking area and avoid the creation of hot or cold spots. The forced air of a convection oven cuts down on overall cooking time, and also allows roasted foods to retain more moisture by moving fast hot air past the food.

Another form of a convection oven is impingement oven. Impingement ovens employ a high flow rate of hot air from both above and below the food. The air flow is directed through nozzles or slots onto food which usually passes through the oven on a conveyor belt. Impingement ovens can achieve much higher heat transfer coefficient than can a conventional (conduction) oven. They are widely used in pizza, cookie, and biscuit

baking. The air-jet impingement oven consists of multiple jet nozzles through which the high velocity air is forced perpendicularly against the baking product.

Figure 4. Heat transfer of natural convection oven, forced convection oven, and impingement oven (Adapted from Walker 1987)



It has been demonstrated to be able to transfer heat more rapidly at lower temperatures and shorter baking times, producing products with higher moisture contents, slower staling rate the products aren't energy efficient. (Pylar 1988, Varilek and Walker 1984, and Vidal and Walker 1995).

## **CHAPTER 2 - Total anthocyanin and dietary fiber contents remaining in blue corn cookies as affected by the baking formula ingredients**

Based on all the information above, blue corn has potential health-beneficial components that are temperature & pH determined. So this study is focused on how the ingredient changes affect anthocyanin and dietary fiber contents and other cookie quality (Chapter II) and how the baking process (baking oven varieties) and pH change (acidulant varieties) affect the blue corn cookies (Chapter III).

### **Objectives & hypothesis**

The hypothesis of this research is that varying ingredients (blue corn flour%, guar gum%, and water%) will result in an increased retention of anthocyanin in blue corn cookies.

1. To determine the effect of blue corn content on the total anthocyanin content (TAC) in cookies containing whole grain blue corn flour and guar gum;
2. To investigate the effect of guar gum & blue corn on the total dietary fiber (TDF) in cookies;
3. To investigate the relation between targeted formula changes and cookie quality indices.

### **Materials & Methods**

Commercially grown blue corn (donated by Sunny State Products, San Jon, NM) was milled into whole grain blue corn flour by hammer mill (Bliss Eliminator, Bliss Industries, Inc., Ponca City, OK) in the milling lab of the Grain Science Department, Kansas State University. The blue corn kernels were milled through #4 (1580  $\mu\text{m}$ ) and #0.4 (160  $\mu\text{m}$ ) screens and followed by sifting (through 50 GG/355  $\mu\text{m}$ , 68 GG/240  $\mu\text{m}$ ,



and pan) (laboratory box sifter, Great Western MFG Co. Inc., Leavenworth, KS) each time. The stream above the 50 GG/355  $\mu\text{m}$  screen was milled again through the laboratory Ross mill with smooth rolls (gap feeler gauge 0.076 mm) (Ross Machine & Mill Supply, Oklahoma City, OK) and followed by sifting. The stream through 70 GG (on pan) was considered corn flour (personal communication with experienced miller Ron Stevenson and Milling Professor Dr. Dale Eustace). Blue corn kernels' test weight, moisture, protein, starch, and density (Table 1) were measured by Test Weight Apparatus (Burrows Equipment Co., Evanston, IL) and Grainspec (Foss Electric, San Antonio Texas). The wheat pastry flours were donated from ConAgra Foods (Omaha, NE). Both flours' moisture, protein, ash, and fat (wet basis) were measured (Table 2) (AACC 2000).

Table 1. Proximate analysis and physical properties of blue corn kernels

moisture	protein	starch	density	Test weight
%	%	%	%	lb/bu
5.28	6.33	79.50	1.25	56.1

Table 2. Proximate compositions of pastry and whole grain corn flours

	moisture%	protein%	ash%	fat%
		(14% mb)	(14% mb)	(wet basis)
pastry flour	12.82	7.88	0.48	0.61
whole grain corn flour	12.88	6.08	0.96	2.88

Whole grain corn flour and wheat pastry flour were dry-blended at three levels of corn flour supplementation (Table 3) based on preliminary tests. The blended flours were used to make sugar snap cookies (AACC method 10-50D). Guar gum (Tic Gums, Belcamp, MD), was added to the cookie formula at various levels to increase the water retention capacity and dietary fiber content (Ward and Andon 1993). Cookies' spread was also measured and calculated (AACC method 10- 50D).

The adjusted blue corn cookie formula is given below. The “total flour” was a blend of whole grain blue corn flour and wheat pastry flour at various ratios. The dough was baked at 400°F for 10 min as specified by AACC method 10-50D.

Shortening	64g
Sugar	130g
Salt	2.1g
Sodium bicarbonate	2.5g
Glucose	33g
Guar gum	variable
Water (distilled)	variable
Total flour (14mb)	225g

Table 3. Response Surface Methodology (RSM) design layout for three-variable bake tests with 11 runs

RSM#	CORN%	GUM%	WATER%
1	0	1	18.5
2	0	0	21.5
3	0	0.5	24.5
4	40	0	18.5
5	40	0.5	21.5
6	40	0.5	21.5
7	40	0.5	21.5
8	50	1	24.5
9	80	0.5	18.5
10	80	1	21.5
11	80	0	24.5

Note: Conditions of RSM #2 were the control values.

Table 4. RSM experimental design for blue corn cookies

	Water% <sub>L</sub>			Water% <sub>M</sub>			Water% <sub>H</sub>		
	Gum% <sub>L</sub>	Gum% <sub>M</sub>	Gum% <sub>H</sub>	Gum% <sub>L</sub>	Gum% <sub>M</sub>	Gum% <sub>H</sub>	Gum% <sub>L</sub>	Gum% <sub>M</sub>	Gum% <sub>H</sub>
<b>Corn%<sub>L</sub></b>			<b>1</b>	<b>2</b>				<b>3</b>	
<b>Corn%<sub>M</sub></b>	<b>4</b>				<b>5,6,7</b>				<b>8</b>
<b>Corn%<sub>H</sub></b>		<b>9</b>				<b>10</b>	<b>11</b>		

Notes:

1. Water%<sub>L</sub>, Water%<sub>M</sub>, and Water%<sub>H</sub> stand for low water level (18.5%), medium water level (21.5%) and high water level (24.5%);
2. Gum%<sub>L</sub>, Gum%<sub>M</sub>, and Gum%<sub>H</sub> stand for low guar gum level (0%), medium guar gum level (0.5%), and high guar gum level (1%);
3. Corn%<sub>L</sub>, Corn%<sub>M</sub>, and Corn%<sub>H</sub> stand for low blue corn flour level (0%), medium blue corn flour level (40%), and high blue corn flour level (80%).

Total anthocyanin content (TAC) was measured according to the modified method of Abdel-Aal and Hucl (1999), with Cyanidin 3-glucoside (Polyphenols Laboratories, Sandens, Norway) was used as a standard. A 3 g sample of the baked cookie was ground in a Waring blender (Dynamics Corporation of America, New Hartford, CT) (where flour sample was weighed directly) and then weighed into a 15-ml centrifuge tube with 10 ml acidified methanol solution (methanol and HCl 1.0 N, 85:15, v/v) was added to the tube. The mixture was adjusted to pH 1 with HCl (10 N) then shaken in an oscillating shaker (Eberbach Co, Ann Arbor, Michigan) for 30 min (check and readjust to pH 1 during the first 15 min if necessary). The tube was centrifuged at 2060 ×g for 15 min. Absorbance of supernatant (U-2010 spectrophotometer, Hitachi, Japan) was read at 535 nm against a reagent blank. Standard curve was made separately.

Total dietary fiber (TDF) was measured following the Total Dietary Fiber Assay Procedure (AOAC 2000) using a TDF assay kit from Megazyme (Megazyme International Ireland Ltd., Bray, Ireland).

A three-point break test (triple-beam snap test) by TA.XT2 Texture Analyzer (Texture Technologies, Scarsdale, NY) was adapted from Hix et al (1997) to measure the texture of the cookies. The settings were:

Force sensitivity	5g
Distance:	50% strain
Pretest speed / test speed / post test speed:	5/1/10 mm/sec
Measure:	peak force

All tests were performed in triplicate on whole cookies. The Surfscan Image-analyzer Program (AEW Consulting, Lincoln, NE) was used to analyze the cookies' brightness, crack ratio, diameter, and eccentricity.

Response Surface Methodology (RSM) was used as the experimental design on a regression / correlation analysis as the statistical tool. Table 4 presents the design for an incomplete block design using 3 independent variables (blue corn flour%, guar gum%, and water%) at 3 levels each. Duplicates of the block were baked on different days. A total of 11 trials with 3 center points (RSM #5, 6, and 7) were generated each day. Table 3 lists the levels for each of the three variables in the formula for each block. Response variables included TDF, TAC, spread, brightness, surface cracks, diameter, and eccentricity.

RSMPlus software (Walker and Walker 1992) was used for data analysis. The following second-order multiple regression equation was generated and fitted to the data (equation 1):

$$\begin{aligned} \text{Dependent Variable} = & A + B*\text{Corn}\% + C*\text{Gum}\% + D*\text{Water}\% + \\ & E*\text{Corn}\%*\text{Gum}\% + F*\text{Gum}\%*\text{Water}\% + G*\text{Corn}\%*\text{Water}\% + \\ & H*(\text{Corn}\%)^2 + I*(\text{Gum}\%)^2 + J*(\text{Water}\%)^2 \end{aligned} \quad (1)$$

Corn% stands for whole grain blue corn flour% in the corn and pastry flour blend; Gum% stands for guar gum% (fwb); Water% stands for water% added in the cookie dough; R is any of the response variables mentioned above and the coefficients A, B, C,

D, E, F, G, H, I, and J are empirical constants generated by the RSM software. Regression models were evaluated based on the multiple correlation coefficient ( $r^2$ ).

## Results and Discussion

### Total anthocyanin content (TAC)

The TAC of raw blue corn flour and wheat pastry flour were 348.3 and 28.2 mg/kg, respectively. The contour map of the response TAC in blue corn cookie is presented in Figure 4. It demonstrates a linear relation to the percentage of blue corn flour (fwb) in the formula at each water level (18.5, 21.5, and 24.5%) (fwb). The letters “A, B, ..., J” in Figure 4 indicate different levels of TAC predicted under the combinations of blue corn flour%, guar gum%, and water%. Thus the higher the blue corn flour% in the formula, the higher the TAC that remained in the cookies. In order to reach highest anthocyanin content (99mg/kg, “J” area in the figure), blue corn flour should be at least at a level of 80% of the flour total and a water level of 18.5% (Figure 4). TAC in blue corn cookies was slightly higher at the higher water levels (21.5% and 24.5%), which might be explained by that a higher moisture content results in lower cookie internal temperature, thus less thermal degradation of anthocyanin.

The regression equation (2) for the results shows the first order, second order, and their interaction relationships for the three independent variables – blue corn flour%, guar gum%, and water% on TAC. The coefficient of determination  $R^2$  is 0.99. If given the concentration of corn%, gum%, and water%, TDF can be predicted for any combination, whether or not it was actually tested.

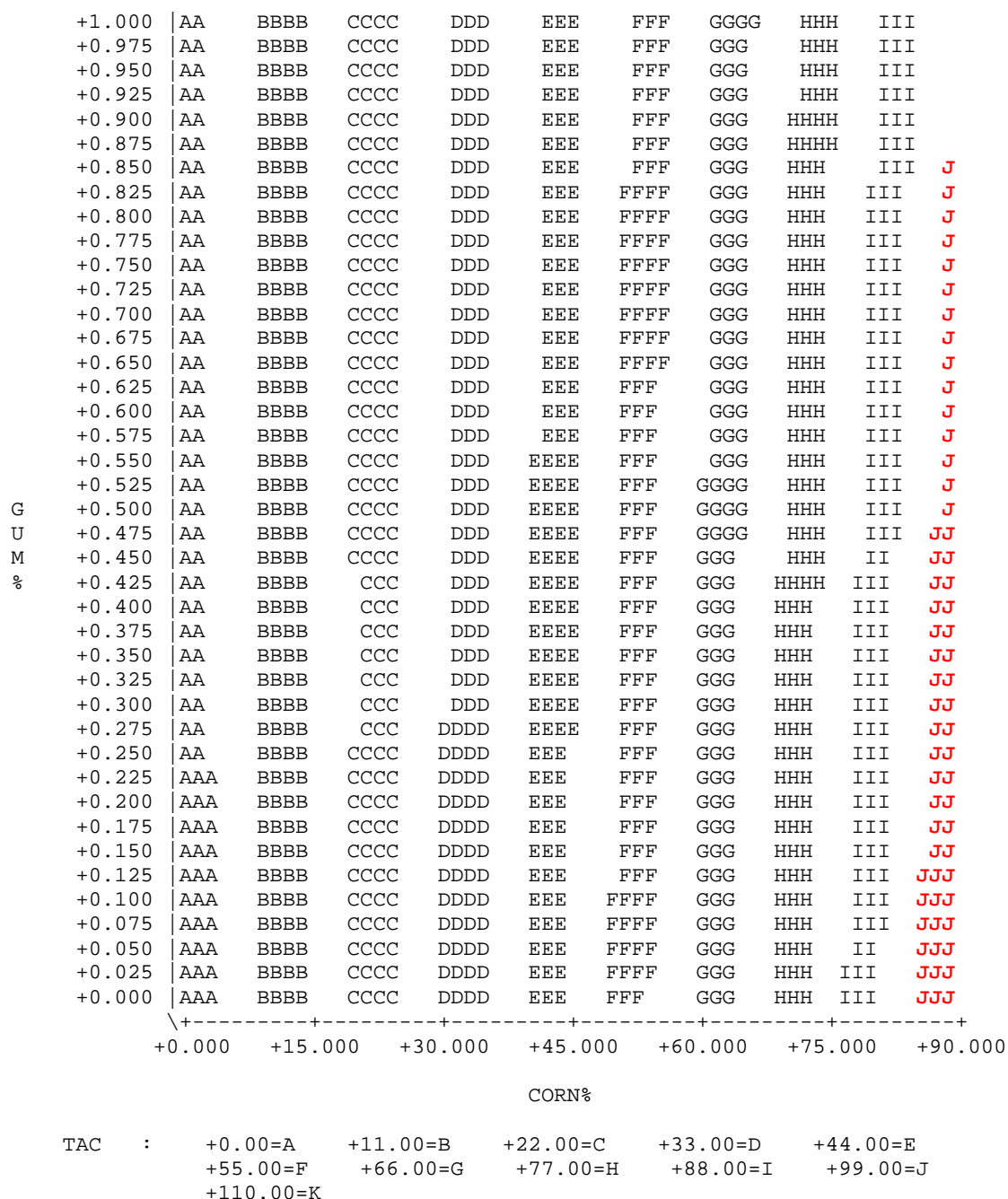
$$\begin{aligned} \text{TAC} = & -43.28 + 0.70 * \text{CORN\%} + 25.72 * \text{GUM\%} + 3.85 * \text{WATER\%} \\ & -0.079 * \text{CORN\%} * \text{GUM\%} -1.32 * \text{GUM\%} * \text{WATER\%} + 0.015 * \text{CORN\%} * \\ & \text{WATER\%} \\ & + 0.0019 * (\text{CORN\%})^2 + 0.41 * (\text{GUM\%})^2 - 0.075 * (\text{WATER\%})^2 \end{aligned} \quad (2)$$

Some of the anthocyanins were destroyed during the cookie baking process for variable combinations (Table 5). Because the majority of anthocyanins are present in blue corn flour rather than in the wheat pastry flour, there is more TAC remaining in the cookies containing a higher percentage of blue corn flour. It might be that wheat anthocyanin is more susceptible to thermal degradation than are the corn anthocyanins. Compared to the studies of anthocyanins during nixtamalization , tortilla baking, and tortilla chip frying, where losses were 37%, 54%, and 75%, respectively (Del Pozo-Insfran et al 2006), anthocyanin loss is lower in the cookie baking process, which is longer in time but a lower processing temperature, and a milder pH change (see Chapter 3).

Table 5. TAC loss in blue corn cookies during baking

blue corn flour %	TAC in cookie dough mg/kg	TAC in cookies mg/kg	TAC loss %
0	12.97	3.87	70.16
40	74.08	44.85	39.46
80	131.48	94.53	28.10

Figure 5. Response surface for TAC in blue corn cookie as affected by guar gum% and blue corn flour% (Stepping variable water% = 18.5%)  $R^2 = 0.99$



### **Total dietary fiber (TDF)**

The TDF of raw whole grain blue corn flour and wheat pastry flour were 4.7 and 3.07 g/100g. RSM analysis displayed a contour map. TDF in blue corn cookies was affected by guar gum% and blue corn% at all three water levels (Figure 6, Appendix A.3, and A.4). At a water level of 18.5%, guar gum and blue corn flour content need to be 0.375 and 90%, respectively, to achieve the TDF of 3.2 g/100g (“G” area in the figure). If the blue corn flour was reduced to 75%, guar gum had to be present 1% in order to reach the same TDF content at the same water level (Figure 6).

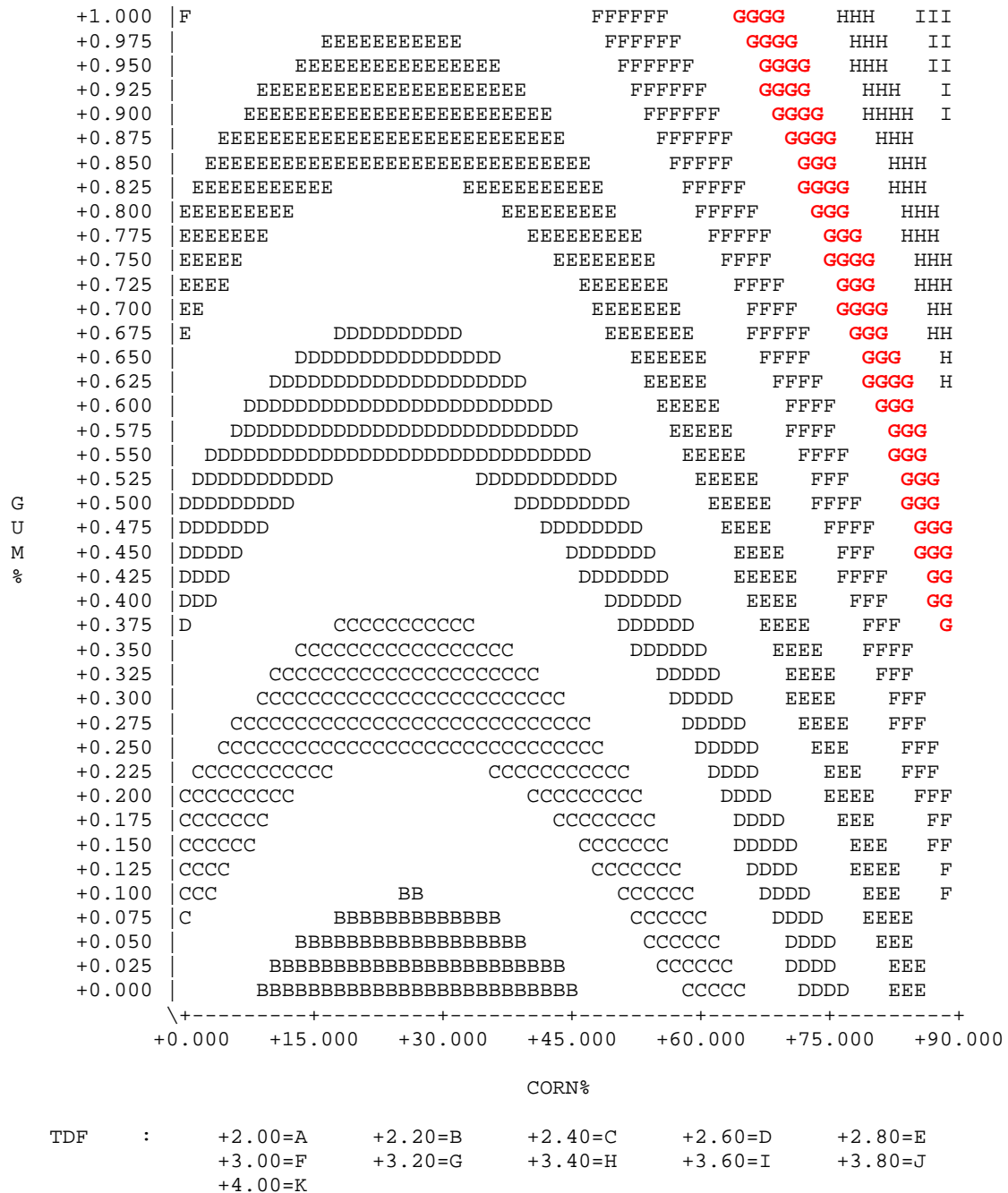
The equation (3) created by RSMPlus (Walker and Walker1992) shows the first order, second order, and their interaction relationships for the three independent variables – blue corn flour%, guar gum%, and water% on TDF. If given the concentration of corn%, gum%, and water%, we can predict TDF for any combination, whether or not it was actually tested. The coefficient of determination  $R^2$  was 0.63.

$$\begin{aligned} \text{TDF} = & 1.06 + 0.0046 * \text{CORN\%} + 1.31 * \text{GUM\%} + 0.063 * \text{WATER\%} \\ & + 0.0010 * \text{CORN\%} * \text{GUM\%} - 0.035 * \text{GUM\%} * \text{WATER\%} - 0.00079 * \text{CORN\%} * \\ & \text{WATER\%} \\ & + 0.0019 * (\text{CORN\%})^2 - 0.016 * (\text{GUM\%})^2 + 0.00038 * (\text{WATER\%})^2 \end{aligned} \quad (3)$$

The formula combination 80% blue corn flour, 1% guar gum, and 21.5% water was found to contain 3.4 g TDF/100g. The average cookie weight in this study was 24g. By calculation there was 0.82 g TDF per cookie. A serving of 3 cookies contains 2.45g TDF, which is more than 10% of the NCI (National Cancer Institute)’s recommended daily level (20-35 g per day) (Anon 2008). Therefore, this type of blue corn cookie could be claimed to a “good source” of dietary fiber.



Figure 6. Response surface for TDF in blue corn cookie as affected by guar gum% and blue corn flour% (Stepping variable water% = 18.5%)  $R^2 = 0.63$



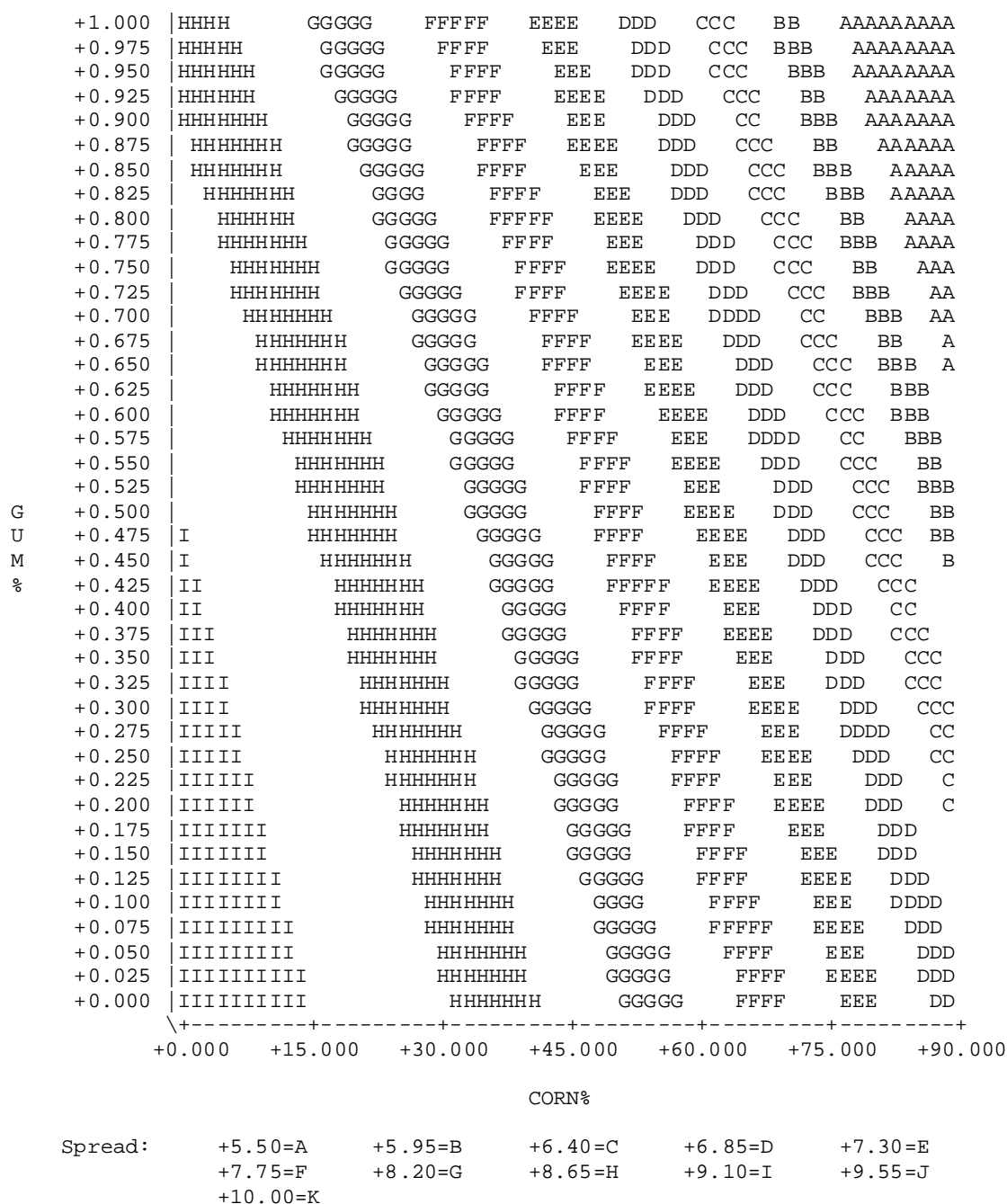
## Cookie spread

Cookie spread is associated primarily with the hydration capacity of flours. It can be explained by the volume of aqueous phase in the dough system (Pylar 1988). Whole grain blue corn flour contains higher amounts of hemicellulose, brans, and germ compared with refined flour because non-endosperm portions were still in the flour. Guar gum has high absorption capacity as well. All these components absorb water in the cookie dough system. That should reduce the volume of the aqueous phase, thus, reducing the cookie spread. This was confirmed by the RSM analysis (Figure 7, Appendix A.5, and A.6), which indicated that cookie spread had an inverse linear relation with blue corn% and guar gum% of all three water levels. The amount of water had a greater effect on spread than did the other two factors affecting the spread. Equation (4) created by RSMPlus shows the first order, second order, and their interaction relationships for the three independent variables – blue corn flour%, guar gum%, and water% on cookie spread. The coefficient of determination  $R^2$  is 0.86. If given the concentration of corn%, gum%, and water%, we can predict TDF for any combination using this equation, whether or not it was actually tested.

Spread =

$$\begin{aligned} & 9.26 - 0.0066 * \text{CORN\%} + 0.077 * \text{GUM\%} - 0.097 * \text{WATER\%} \\ & - 0.018 * \text{CORN\%} * \text{GUM\%} - 0.0039 * \text{GUM\%} * \text{WATER\%} + 0.00022 * \text{CORN\%} * \\ & \text{WATER\%} \\ & - 0.00026 * (\text{CORN\%})^2 - 0.42 * (\text{GUM\%})^2 + 0.0050 * (\text{WATER\%})^2 \end{aligned} \quad (4)$$

Figure 7. Response surface for spread in blue corn cookie as affected by guar gum% and blue corn flour% (Stepping variable water% = 18.5%)  $R^2 = 0.86$



## Cookie texture

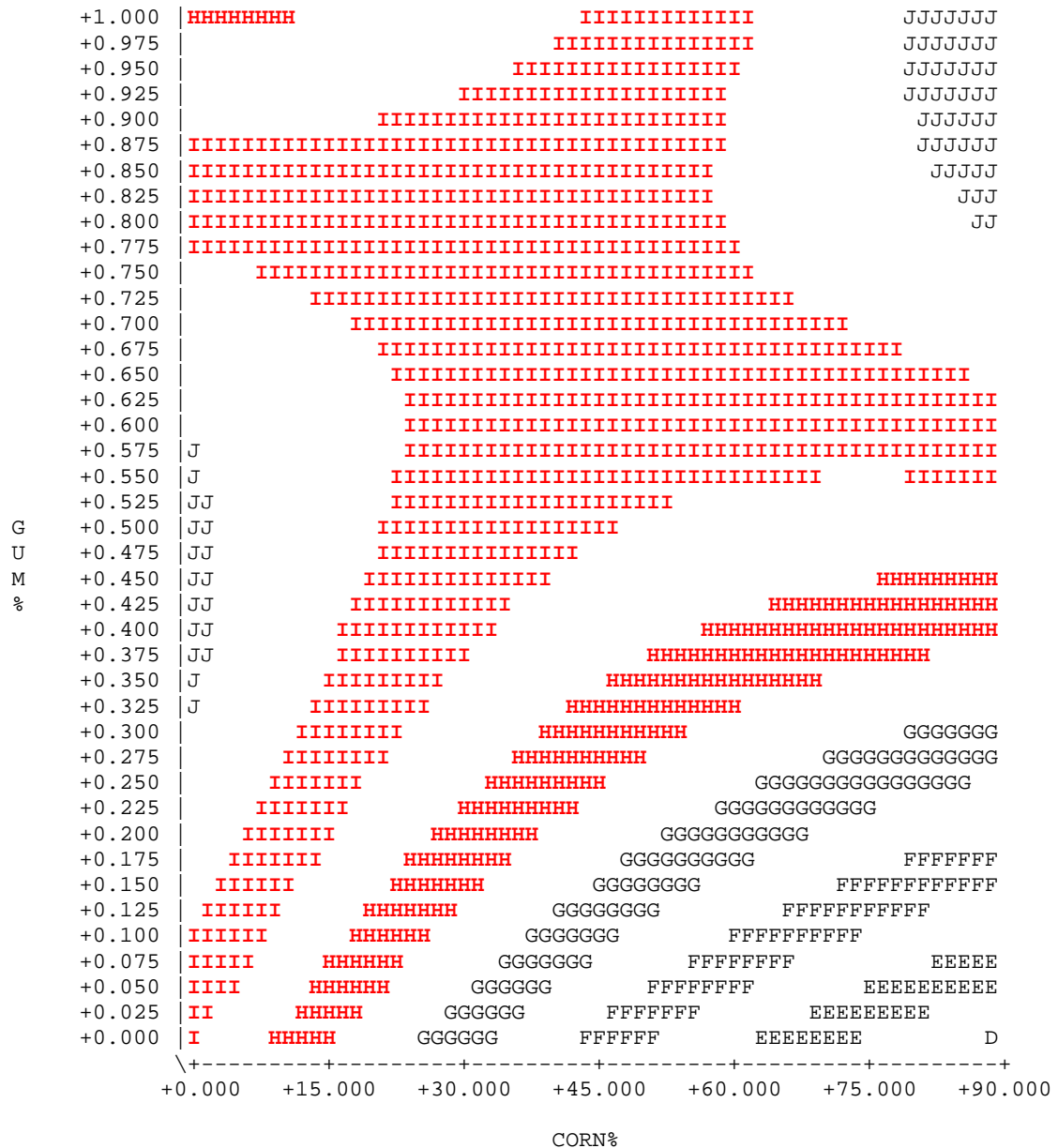
Cookies' softness/hardness was positively associated with the amount of gum and water in the dough (Figure 7). The treatment combination, 0.7% guar gum, 42% blue corn flour, and 18.5% water produced a hardness of 9160g ("I" area in Figure 8). The same treatment but with water increased to 21.5% resulted in lower hardness ("H" area in 8240g) (Appendix A. 7). Presence of guar gum allows more water in the cookie with softer texture (Figure 8, Appendix A.7, and A.8).

Response surface equation 5 shows the first order, second order, and their interaction relationships for the three independent variables – blue corn flour%, guar gum%, and water%, on cookie texture. The coefficient of determination  $R^2$  is 0.87. If given the concentration of corn%, gum%, and water%, we can predict TDF for any combination by using this equation, whether or not it was actually tested.

Cookie texture =

$$\begin{aligned} &6413.10 - 62.48 * \text{CORN}\% + 841.01 * \text{GUM}\% + 674.09 * \text{WATER}\% \\ &+ 67.99 * \text{CORN}\% * \text{GUM}\% + 187.33 * \text{GUM}\% * \text{WATER}\% - 0.040 * \text{CORN}\% * \\ &\text{WATER}\% \\ &+ 0.17 * (\text{CORN}\%)^2 - 4901.06 * (\text{GUM}\%)^2 - 28.22 * (\text{WATER}\%)^2 \end{aligned} \quad (5)$$

Figure 8. Response surface for blue corn cookie texture as affected by guar gum% and blue corn flour% (Stepping variable water% = 18.5%)  $R^2 = 0.87$



## Cookie Brightness

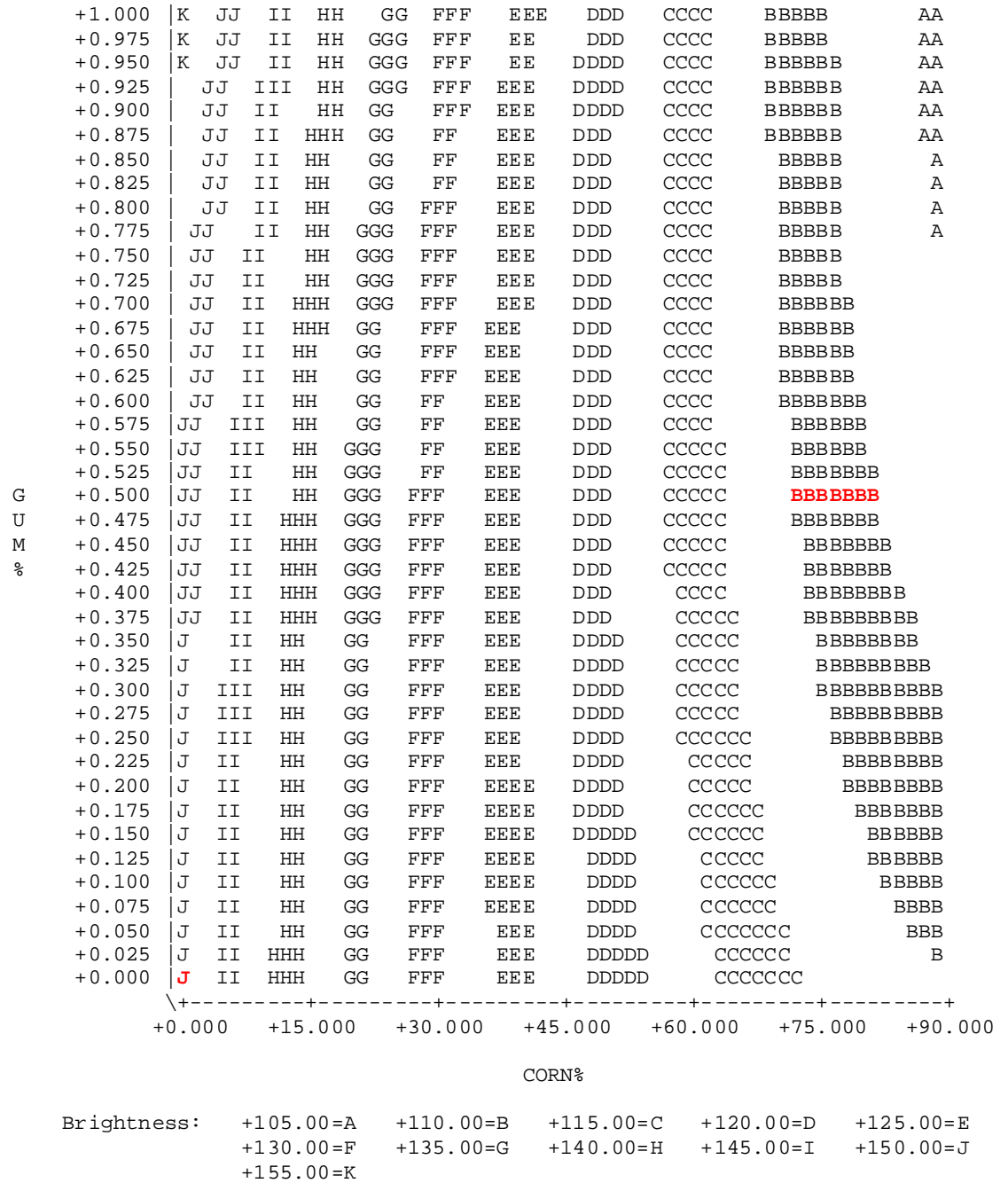
. The surface of cookies containing corn flour was brown after baking as were cookies made from regular pastry flour. However, the blue corn containing cookies' interior was blue due to the pigment present in the corn. RSM analysis disclosed a nearly linear relationship between cookie brightness and guar gum% and blue corn% at all three water levels (Figure 9, Appendix A.9, and A.10). Brightness was inversely related to the blue corn flour %. For example, compared to the 100% pastry flour cookies with 21.5% water, 0% guar gum, and 0% blue corn, the brightness at higher blue corn formulas (for example, a water level of 21.5%, guar gum 0.5%, and blue corn flour 80%) was lower compared "B" area to "J" area in Figure 9. The blue pigment in the blue corn cookies makes browning effect more obvious than the regular whitish pastry flour cookies.

The equation (6) created shows the first order, second order, and their interaction relationships for the three independent variables – blue corn flour%, guar gum%, and water% on brightness. The coefficient of determination  $R^2$  is 0.98.

$$\begin{aligned} \text{Brightness} = & 76.86 - 0.61 * \text{CORN\%} + 25.53 * \text{GUM\%} + 5.96 * \text{WATER\%} \\ & - 0.13 * \text{CORN\%} * \text{GUM\%} - 1.10 * \text{GUM\%} * \text{WATER\%} - 0.0070 * \text{CORN\%} * \\ & \text{WATER\%} \\ & + 0.0038 * (\text{CORN\%})^2 + 3.72 * (\text{GUM\%})^2 - 0.12 * (\text{WATER\%})^2 \end{aligned} \quad (6)$$

The cracking pattern on the cookie surface is considered to be the ratio of crack area to "lands" area. The higher the ratio, the deeper and wider the cracks. Eccentricity measures non-uniformity in cookie shape. The higher the eccentricity value, the less uniformly round the shape. Both are considered to be cookie quality factors so they were analyzed. The  $R^2$  for both were low, which indicated that there might be other factors involved, other than water%, corn flour% or gum%.

Figure 9. Response surface for blue corn cookie brightness as affected by guar gum% and blue corn flour% (Stepping variable water% = 21.5%)  $R^2 = 0.98$



## ***Summary of Results***

The TAC of raw whole grain blue corn flour was 348.3 mg/kg. Some anthocyanins were destroyed under the cookie baking conditions of 400 °F for 10 min since the pH, temperature, and moisture changed dramatically. The TAC remaining in blue corn cookie showed a linear relation to the percentage of blue corn flour in the formula at each water level (18.5, 21.5, and 24.5%) (fwb). The higher the blue corn flour% in the formula, the higher the TAC that remained in the cookies. TAC loss during baking decreased with the increase of blue corn flour level in the formula. When the blue corn flour was added at 80% (fwb), the TAC loss was only 28.10%. Compared to the anthocyanin loss in nixtamalization, tortilla baking and tortilla chip frying (Parra et al 2007), cookie baking is a milder process, and more TAC remains in the final products.

The TDF was found to show a second order relation with blue corn flour%, guar gum%, and water%. Guar gum has more influence than other factors due to its high fiber concentration, even if it is only added up to 1% (fwb). The blue corn cookies with 80% blue corn flour, 1% guar gum, and 21.5% water contained 2.61g TDF per serving (if one serving is defined as three cookies with average weight of 24 g each). This formula can be considered to be a “good source” of dietary fiber according to the standard of FDA regulations.

Blue corn cookie spread was found proportional to water % in the formula, which weighs heavier than the other two factors. Increased levels of blue corn flour and guar gum reduced the cookie spread. Cookies’ softness/hardness was associated with the amount of gum and water in the dough. The addition of guar gum allows more water in the cookie, with a softer texture. The brightness was inversely related to the blue corn flour %.

Based on the previous discussion and baking experience, the formula of 80% blue corn flour, 1% guar gum, and 21.5% water was used in the next part of study.

## ***Conclusions***

It was found that varying ingredients (blue corn flour%, guar gum%, and water%) will result in an increased retention of anthocyanin in blue corn cookies.



## **CHAPTER 3 - Total anthocyanin content in blue corn cookies as affected by the various acids and oven types**

### **Objectives**

The hypothesis of this research is that varying oven types and acidulant agents will result in increased retention of anthocyanin in blue corn cookies because oven heat transfer rate and pH are key factors to influence the retention of anthocyanins.

1. To determine the appropriate baking conditions (baking time and temperature) of convection, impingement, and reel ovens and the appropriate level of each acid (citric acid, lactic acid and glucono-delta-lactone (GDL)) in order to retain maximum anthocyanin content for blue corn cookies by single factor experiments.
2. To study the interactions of ovens and acids on TAC (total anthocyanin content), cookie spread, texture, and other quality indices of blue corn cookies by RSM.
3. To select the best combination of oven (reel, convection, and impingement oven) baking conditions and acid choice (citric acid, lactic acid, and GDL) & quantity on the anthocyanin content in the blue corn cookie.

### **Materials and Methods**

Whole grain blue corn was milled into flour according to the method described in Chapter two. The wheat pastry flour was the same as used in the previous tests.

Citric acid, lactic acid, and GDL were purchased from Fishers scientific (Fair Lawn, New Jersey), Purac (Gorichem Netherlands), and Sigma-Aldrich (Saint Louis, MO, USA), respectively.

Reel oven (Despatch Minibake, Despatch oven Co. Minneapolis, MN ), convection oven (SunFire® Garland Commercial Industries Inc., Freeland,

Pennsylvania), and impingement oven (Middleby Marshall Electric, Pacesetter, Model PS 200, Morton Grove, Illinois) were used to bake cookies under their optimum baking conditions.

### ***Acid addition***

The formula for blue corn cookies was based on the formula described in Chapter two, where the ratio of whole grain blue corn flour and wheat pastry flour was set at 80:20, and guar gum was added at 1%, based on the preliminary tests. Various acids (citric, lactic, or GDL ) were added alone at 0, 1, 2, 4, or 6% (fwb) to observe the pH change in the dough and cookie. Baking conditions were fixed (AACC 10-50D) at 400°F (204.4 °C) for 10 min in the reel oven. TAC and cookie spread, texture, brightness, surface cracks, and eccentricity were measured according to the method described in Chapter two. Cookie moisture was measured according to AACC method 44-15A. The pH of the dough and cookies were recorded right after mixing and the day after baking, respectively. The level of each acid which retained maximum TAC was selected.

### ***Oven type***

The formula for blue corn cookie based on the formula described in Chapter two, where the ratio of whole grain blue corn flour and wheat pastry flour was set at 80:20, and gum was added at 1%. Acids were not added. Appropriate baking temperatures and times were decided by running a series of tests on each of the reel, convection, and impingement ovens. The baking time for each temperature was decided when the cookies were baked lightly brown on the bottom and edge. RSM (response surface methodology) was used as a statistical tool for each oven test. Tables 6, 7, and 8 present the incomplete block design using two independent variables (time and temperature) at three levels, low, medium, and high. Duplicates of the blocks were baked on different days. Response variables included TAC, spread, brightness, and surface cracks. The optimum baking time and temperature of each type for oven was selected based on maximum retention of TAC and optimum cookie quality (spread, texture, brightness, and etc.) from reel oven as a control.

Table 6. Response Surface Methodology (RSM) experimental design for reel oven

Sample#	Baking temperature	Baking time (min)
1	176.7C (350F)	13
2	176.7C (350F)	16
3	204.4C (400F)	10
4	204.4C (400F)	10
5	204.4C (400F)	10
6	232.2C (450F)	7
7	232.2C(450F)	10

Table 7. RSM experimental design for forced convection oven

Sample#	Baking temperature	Baking time (min)
1	176.7C (350F)	7
2	176.7C (350F)	9
3	187.8C (370F)	5
4	187.8C (370F)	5
5	187.8C (370F)	5
6	198.9C (390F)	4
7	198.9C (390F)	6

Table 8. RSM experimental design for impingement oven

Sample#	Baking temperature	Baking time (min)
1	171.1C (340F)	7
2	171.1C (340F)	9
3	182.2C (360F)	5
4	182.2C (360F)	5
5	182.2C (360F)	5
6	193.3C (380F)	4
7	193.3C (380F)	6

### ***Interaction of acid addition and oven type***

The interaction of the appropriate baking conditions for each type of oven and acid combinations were conducted as an incomplete block described in Table 9.

Duplicates of the block were run on different days. pH of each variable's dough and cookie were measured right after mixing and the next day after baking, respectively. TAC, cookie moisture, spread, texture, and crack pattern were measured. ANOVA analysis was used as a statistical tool.

Table 9. Experimental design for interaction of acid addition and oven type

Variable#	Oven	Baking temperature (°F)	Baking time (min)	Acid type	Acid (mol/kg flour)
1	reel oven	204	10	lactic acid	0.17
2	convection	188	5	lactic acid	0.17
3	impingement oven	182	5	lactic acid	0.17
4	reel oven	204	10	GDL	0.086
5	convection oven	188	5	GDL	0.086
6	impingement oven	182	5	GDL	0.086
7	reel oven	204	10	citric acid	0.076
8	convection oven	188	5	citric acid	0.076
9	impingement oven	182	5	citric acid	0.076

## Results and Discussions

### *Acid addition*

Acidulants were added to lower the pH in the cookies and protect the anthocyanins against decomposition. Table 10 shows TAC, moisture, spread, and pH in blue corn dough and cookies with the supplementation of GDL, lactic acid, and citric acid at 0-6% (fwb). The acidification in the cookies didn't have a major effect on the cookies' moisture content or spread (Table 10).

The cookies with acids appeared visually to be lightly pink as compared to the brown on the surface and blue in the interior of the cookies made without extra acid added. This resulted from the supplementation with different levels of acids, which confirmed the mechanism of anthocyanins' stability (Francis 1989) (Figure 2). The blue corn cookies were brown on the surface because baking at high temperatures favors the formation of the chalone C, with a resulting loss in color. This reaction is considered to be the degradation path (Brouillard, 1982). However, cooling and acidification may drive the blue/pale purple quinoidal base (A) and colorless carbinal base (B) back to the red cationic form  $AH^+$ , which predominates in terms of visual color (Francis 1989).

The addition of acids lowers the pH in both dough and cookies. The more acids were added, the lower the pH. Figures 9, 10, and 11 also show that TAC dramatically increased with the addition of each acid at all levels and the increase also reached a

plateau at about 1.5-2% acid. Citric acid, lactic acid, and GDL are all weak organic acids. Citric acid, with three replaceable hydrogens contains the strongest degree of sourness among these three acids (Watine 1995). The more free anions that are associated with an acid, the more anthocyanin retention in the lower pH cookies. The cookies containing more than 4% citric acid and lactic acid tasted obviously sour and unpleasant. The pH of these cookies was below 4.33. Lactic acid supplementation also brings a distinct “sour dough” smell, which may not be popular in cookies, unlike in breads.

Figure 10 displayed the effects of each acid on cookie surface appearance. All the acid supplements brought decent crack patterns. With higher level of each acid added, the cookies showed pinker rather than brown, which indicated more anthocyanin remained after baking in the cookies. This trend also confirmed with the results of TAC measurement (Table 10).

TAC was considered the predominant factor to use in selecting the optimum level for each acid. According to Figures 11, 12, and 13, it is obvious that the level of about 1.5% (fwb) is enough to retain maximum TAC in the blue corn cookies, which is 0.17, 0.086, and 0.076 mol/kg flour for lactic, GDL, lactic acid, respectively, respectively, without resulting in an excessively sour taste.

Table 10. The effect of acid supplementation on total anthocyanin content (TAC), dough pH, cookie pH, moisture% and spread of blue corn cookies

	acid%	TAC (mg/kg)	dough pH	cookie pH	moisture%	spread
Lactic acid	0	101.3±2.7	7.5	8.8	4.4	6.7
	1	214.1±3.0	6.6	7.1	4.5	6.7
	2	235.3±2.1	5.8	5.7	4.7	6.8
	4	237.2±4.4	4.3	4.4	4.3	6.6
	6	226.7±2.8	4.0	4.1	4.7	6.5
GDL	0	101.3±2.7	7.5	8.8	4.4	6.7
	1	193.6±0.9	6.9	7.8	4.5	6.9
	2	207.0±6.3	6.6	7.2	4.7	6.6
	4	220.0±2.1	6.4	6.4	4.4	6.8
	6	219.0±2.1	6.3	5.6	4.2	6.8
Citric acid	0	101.3±2.7	7.5	8.8	4.4	6.7
	1	174.9±3.4	5.5	5.7	4.4	6.4
	2	183.3±2.5	4.5	4.6	4.6	6.4
	4	181.5±2.4	3.7	3.8	5.2	6.8
	6	168.5±3.4	3.3	3.4	6.0	6.9

Figure 10. Effects of acids on cookie surface appearance

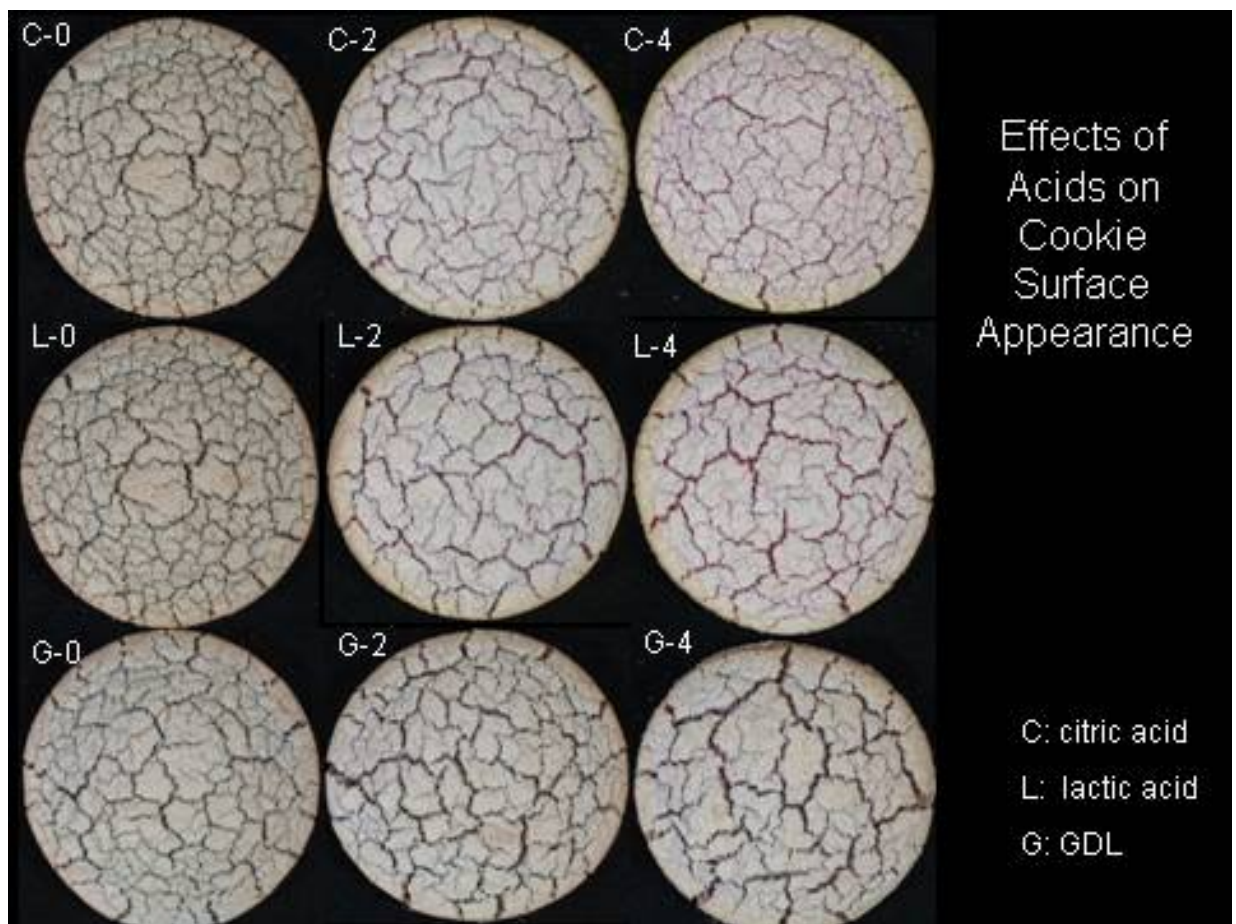


Figure 11. Effect of various levels of GDL on TAC and pH in blue corn cookies

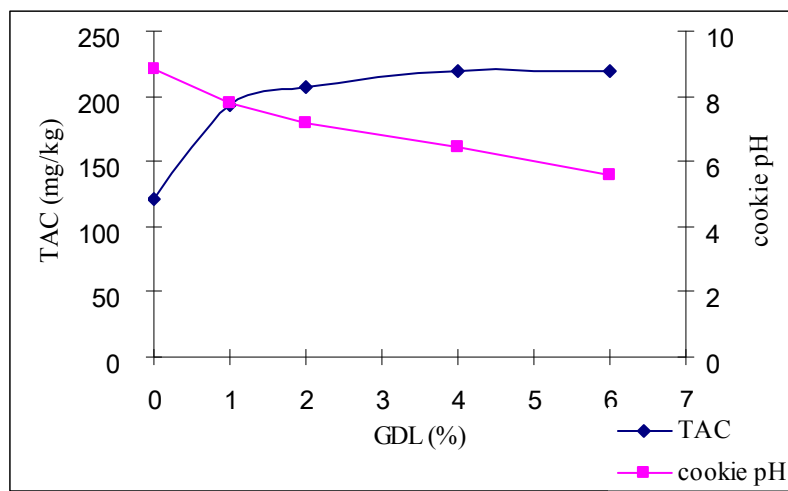


Figure 12. Effect of various levels of lactic acid on TAC and pH in blue corn cookies

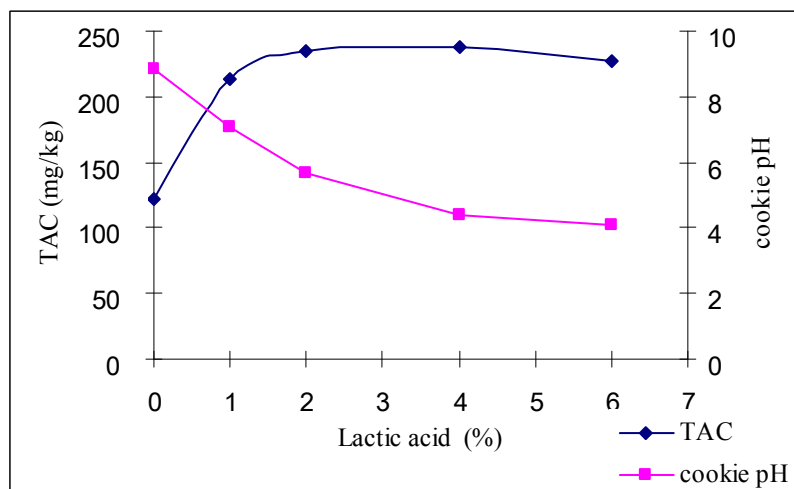
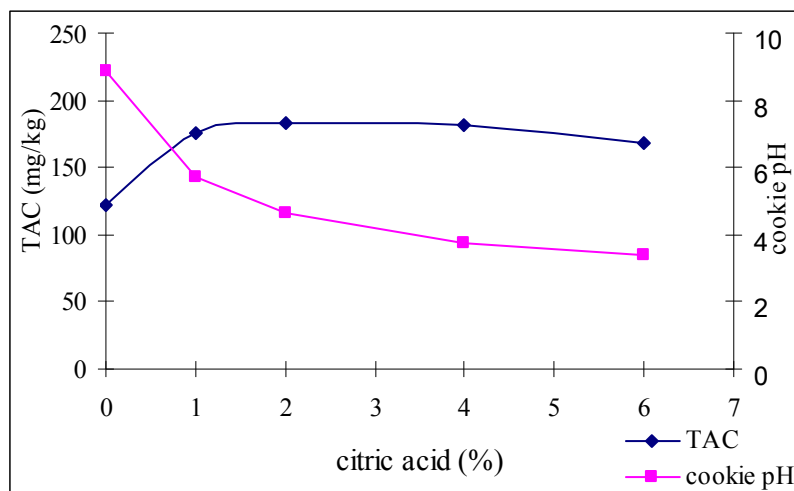


Figure 13. Effect of various levels of citric acid on TAC and pH in blue corn cookies



### ***Oven type***

Natural convection ovens (reel ovens), air forced convection ovens, and air jet impingement ovens have different baking principles (Varilek and Walker 1984), in addition to the traditionally considered terms of time and temperature. The convective heat transfer coefficient is actually another important factor. Table 11 lists the overall heat transfer coefficients (h-value, BTU/HR.FT<sup>2</sup>.F) for various ovens, which were used in this study. It is clear that h-value is the lowest for the reel oven, which indicated relatively poor heat transfer efficiency due to poor air circulation in the oven. This confirms the fact that the reel oven is a natural convection oven, in which currents of hot gases move only slowly through the chamber (Li 1993).

The indirect-fired forced convection oven has burners in a bottom chamber that heats the inner walls. “A fan located at the rear of the baking chamber recirculates the atmosphere but doesn’t draw combustion products into the baking chamber” (Xue et al 2004). The rapid air flow in this type of oven makes the air to constantly recirculate, which results in the heat being transferred to the product more rapidly and the surface moisture is more quickly swept away. These actions increase the heat transfer rate, which means that is possible to use a lower oven temperature without increasing the bake time, or reduced time, or both (Varilek and Walker 1984).



Table 11. h-values for different ovens

	h-value (BTU/HR.FT <sup>2</sup> F)
Natural convection (Reel)oven <sup>1</sup>	3.07-4.10
Indirected fire convection oven <sup>2</sup>	8.35-8.45
Impingement oven <sup>3</sup>	12.18-12.37
Note 1: Data adapted from Li 1993	
2. Data adapted from Xue, Lefort, and Walker 2004	
3: Data adapted from Xue and Walker 2003	

An air-jet impingement oven is a special form of convection oven, in which high velocity air jets are forced perpendicularly against the food product (Dogan and Walker 1999). It has the highest h-value (Table 11), which presents reduction of both baking time and oven temperature. It is generally accepted that foods baked in a typical impingement oven are usually baked at about 20-25 °C lower temperature and about 50-60% shorter time as in a conventional oven, the values vary depending on the product characteristics (Li and Walker 1996). Cookies are thin, low moisture content, and high density and normally require relatively short baking time; therefore the reduction of baking time was reduced by only about 10% in an impingement oven. And most characteristics of cookies baked in an impingement oven are similar to those baked in convectional ovens (Dogan and Walker 1999).

### ***Reel oven***

Figure 12 demonstrates an inverse relation between TAC and baking time/baking temperature. This confirms the theory that anthocyanins are subject to thermal degradation (Francis 1989), where a short time/high temperature combination was also suggested to retain higher anthocyanin content.

The regression equation (7) shows the relationship for two independent variables – baking time and baking temperature, on TAC. The coefficient of determination  $R^2$  is 0.93, which means the equation fits the data very well. Given the combination of baking time and baking conditions, we can predict TAC for any combination, whether or not it was actually tested.

$$\begin{aligned} \text{TAC} = & +339.08 + 3.03 * \text{BAKE TIME} - 0.8 * \text{BAKE TEMPERATURE} \\ & - 0.015 \text{BAKE TIME} * \text{BAKE TEMPERATURE} \\ & - 0.19 * (\text{BAKE TIME})^2 + 0.00082 * (\text{BAKE TEMPERATURE})^2 \end{aligned} \quad (7)$$

Cookie spread was inversely related to baking time (Fig 13). At a baking temperature of 400 °F, cookie spread demonstrated a decrease from 6.4 (“H” area in the figure) to 5.6 (“D” area in the figure) when the baking time increased from 7 min to 15 min. If the blue corn cookies were baked at a short time (7-8.5 min), spread would be predicted to increase from 6.4 (“H” area in the figure) to 6.8 (“J” area in the figure) as the baking temperature increased from 395 °F to 450 °F. This is because the short time but high temperature baking condition allows the cookies’ surface structure to set up rapidly without too much moisture loss as compared to the long baking time treatments. This might help to retain more aqueous phase volume in the cookie dough system center, which increases the spread.

The regression equation (8) for the results shows the relationship for the effects of two independent variables – baking time and baking temperature, on spread. The coefficient of determination  $R^2$  is 0.84. If given the baking time and temperature, we can predict the spread for any combination, whether or not that combination was actually tested.

$$\begin{aligned} \text{SPREAD} = & +11.05 + 0.55 * \text{BAKE TIME} - 0.039 * \text{BAKE TEMPERATURE} \\ & - 0.00097 * \text{BAKE TIME} * \text{BAKE TEMPERATURE} \\ & - 0.012 * (\text{BAKE TIME})^2 + 0.000064 * (\text{BAKE TEMPERATURE})^2 \end{aligned} \quad (8)$$

The data for the response variables for blue corn cookies made by the combination of no acid / 400F / 10 min in a reel oven were used as reference to compare the blue corn cookies made in other ovens. TAC, spread, brightness, and cracks were the response variables used to select the desired baking conditions (temperature and time) for each type of oven. TAC of 103 mg/kg was predicted at the baking conditions of 400F / 10

min in reel oven. In order to retain more TAC,  $TAC \geq 103$  mg/kg (highlighted areas of “H”, “I”, and “J” in red in Figure 12) was considered. Cookie spread from the reel oven (400F / 10 min) was 6.3 (between the area of “G” and “H” in Figure13). The allowance of  $\pm 10\%$ , which is the range of 5.7 to 6.9 in spread, was considered the desired cookie characteristics, which then was used to select the desired baking conditions for all three types of ovens (highlighted areas of “E”, ..., to “J” in red in Figure 13). Cracks ( $R^2 = 0.73$ ) and brightness ( $R^2 = 0.95$ ) data were also analyzed by RSM. The cracks and brightness under the baking conditions of 400 °F and 10 min were 1.25 and 213, respectively (“F” and “H” area in Appendix respectively). The allowance of  $\pm 10\%$ , which is the range of 1.12 to 1.37 in cracks and 191 to 234 in brightness, were considered to represent the best baking conditions for all three types of ovens.

Based on the desired characteristics of cookies and maximum retention of TAC, the combination of baking time of 7-12.4 min and baking temperature of 350-450°F (highlighted area in red) was considered the best for the reel oven (Figure12). A short time/high temperature was considered better to retain the maximum anthocyanin content reported by Francis (1989). Other response variables (brightness, cracks, spread) all overlapped in the acceptable region. Based on the above discussion and baking experience, the baking condition of 400 °F /10 min is the best combination for the reel oven, which is also recommended by the AACC method 10-50D.

Figure 14. Effect of baking time (min) and temperature (F) on TAC of blue corn cookies made in reel oven

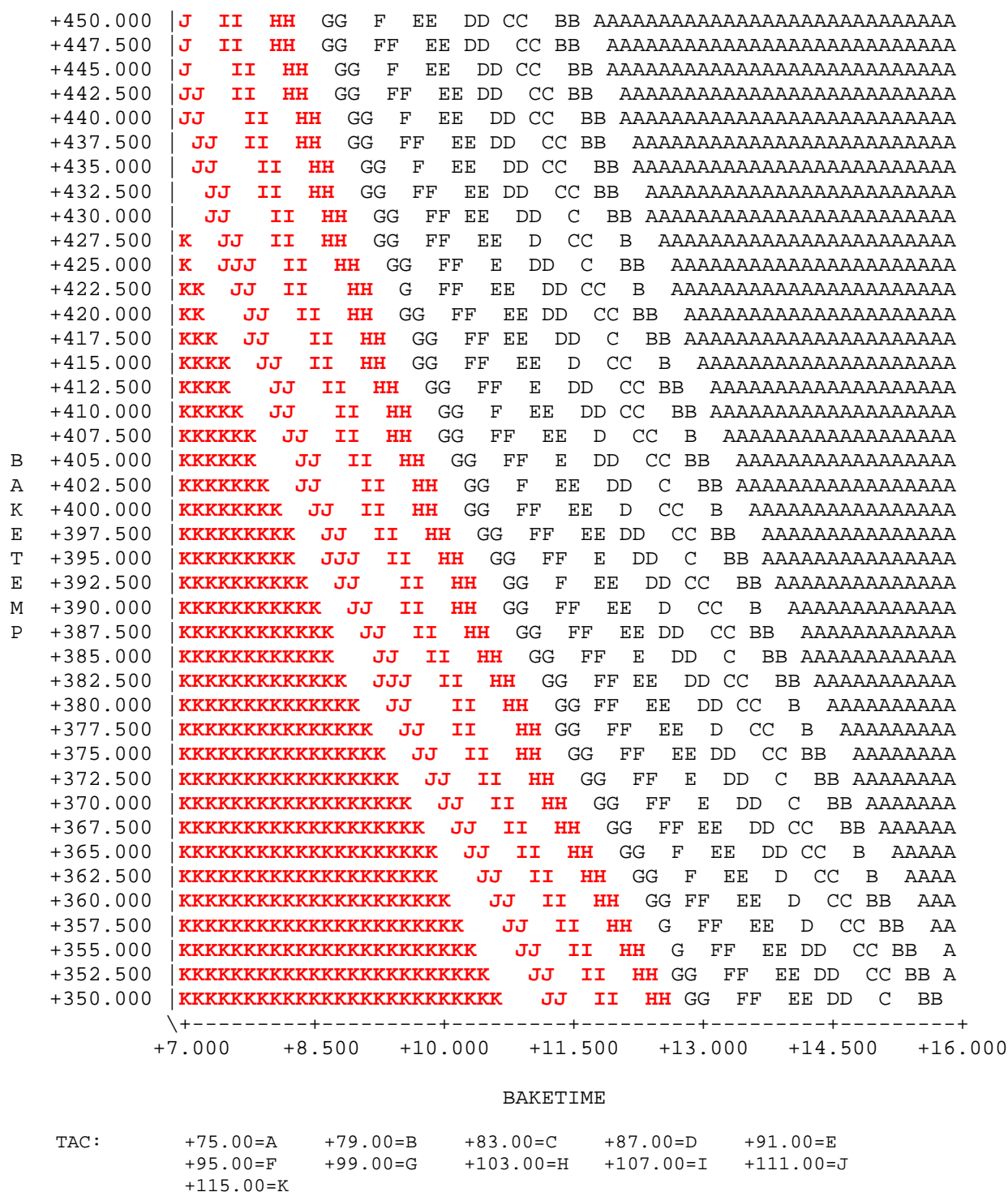
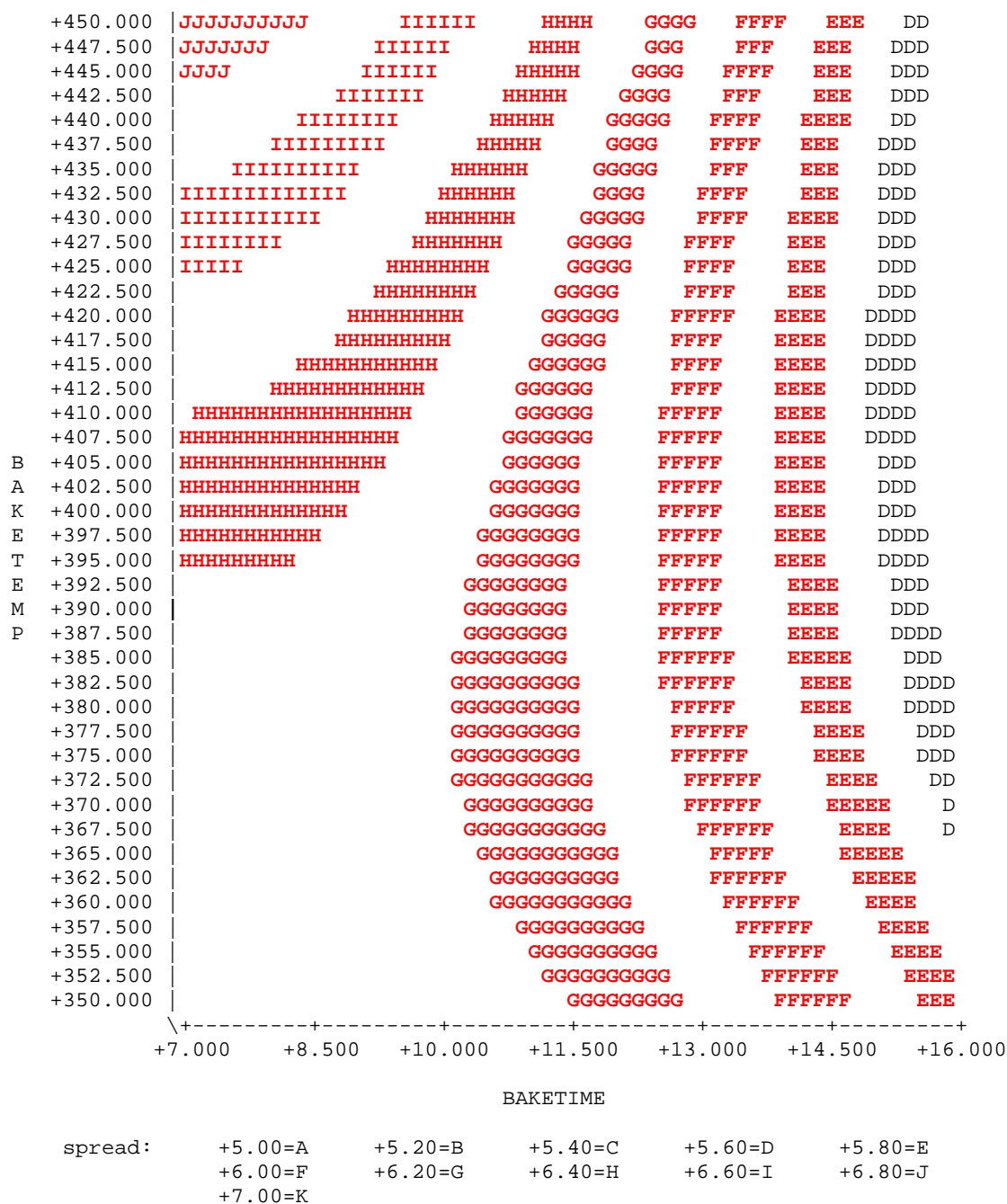


Figure 15. Effect of baking time and temperature on spread of blue corn cookies made in reel oven



Note: BAKTETIME (min), BAKETEMP (F)

### ***Convection oven***

TAC in convection oven - baked cookies demonstrated an inverse relation with baking time and temperature (Figure 15). The regression equation (8) described the effect of all combinations of the independent variables (baking temperature and baking time) on the dependent variable TAC and included their interaction effects.  $R^2$  is 0.93.

$$\begin{aligned} \text{TAC} = & -212.79 + 93.36 * \text{BAKE TIME} + 1.08 * \text{BAKE TEMPERATURE} \\ & -0.26 * \text{BAKE TIME} * \text{BAKE TEMPERATURE} \\ & -0.94 * (\text{BAKE TIME})^2 - 0.000048 * (\text{BAKE TEMPERATURE})^2 \end{aligned} \quad (8)$$

Cookies baked at 390 F for 4 min contained 154 mg/kg TAC; when the cookies were baked at 370 F for 7 min, TAC in the cookies was predicted as 115 mg/kg. This confirms the recommendation of short time and high temperature (Francis 1989) to retain maximum TAC. Generally, the convection oven was more efficient in baking and retaining TAC in the blue corn cookies than was the reel oven. If the cookies were baked at 350F for 7min (130 mg/kg, “F” area in Figure 15) in convection, TAC remaining in the cookies was higher than that at the same baking conditions in a reel oven (115mg/kg, “K” area in Figure 12). But, the cookies were not properly baked under those conditions. This can be explained by the higher heat transfer efficiency in the convection oven which allows more heat-sensitive anthocyanins to survive during the “short time” baked cookies.

Table 11 listed the overall heat transfer coefficients (h-value, BTU/HR.FT<sup>2</sup>.F) for various ovens. It was clear that the h-value is the lowest in the reel oven, which indicates relatively poor heat transfer efficiency due to poor air circulation in the oven. This confirms the fact that the reel oven is a natural convection oven, in which currents of hot gases move slowly through the chamber (Li 1993). The indirect-fired convection oven has burners in a bottom chamber heat the inner walls. “A fan located at the rear of the baking chamber recirculates the atmosphere but doesn’t draw combustion products into the baking chamber” (Xue et al 2004). The rapid air flow in this type of oven makes the air recirculate constantly, which results in the heat being transferred to the product more rapidly and the surface moisture is more quickly swept away. Those increase the baking

rate, which means lower oven temperatures without increasing the bake time (Varilek and Walker 1984).

The relation of cookie spread to baking time / temperature is reported by equation 9 ( $R^2 = 0.87$ ). This empirical model can predict cookie spread for all combinations of the independent variables (baking temperature and baking time). The advantage is that it can be used to predict combinations not actually run.

$$\begin{aligned} \text{SPREAD} = & -155.93 + 7.30 * \text{BAKE TIME} + 0.76 * \text{BAKE TEMPERATURE} \\ & -0.018 * \text{BAKE TIME} * \text{BAKE TEMPERATURE} \\ & -0.080 * (\text{BAKE TIME})^2 - 0.00088 * (\text{BAKE TEMPERATURE})^2 \end{aligned} \quad (9)$$

Based on the reference amount of TAC in reel oven-baked cookies, the baking conditions which result in  $\text{TAC} \geq 103 \text{ mg/kg}$  will be considered as the best baking condition for forced convection oven (red highlighted area in Figure 15). Figure 16 presents the range of baking conditions producing the desired characteristics of cookies in terms of spread (a range of 5.7 to 6.9). Both the combinations of short baking time (3min) and low baking temperature (350F) and long time (9min) and high temperature (390F) can produce a cookie with an acceptable range of spread as defined by the reel oven (“A” area in Figure 16).

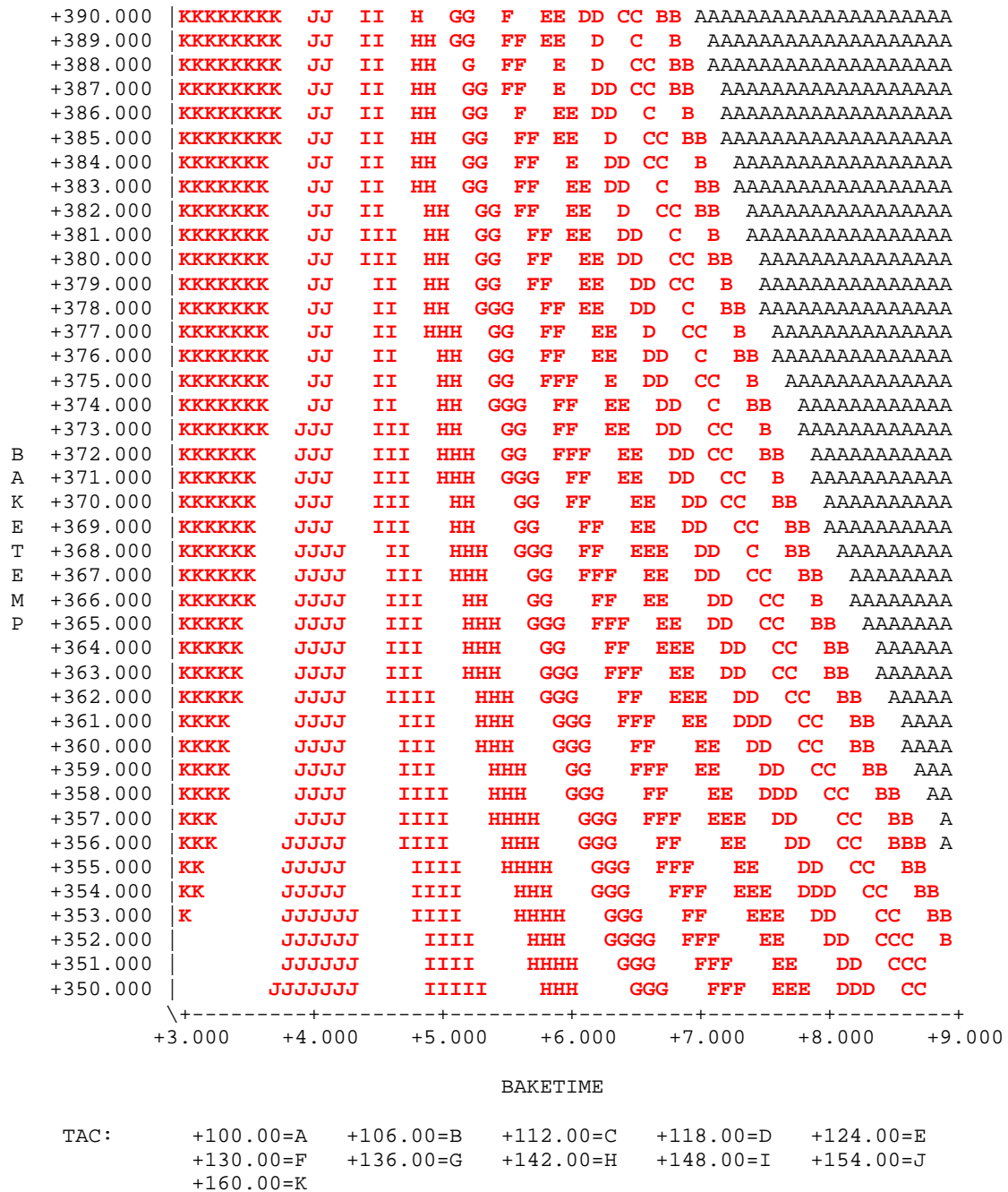
Many other factors still need to be considered to select the best baking conditions. For example, the cookies made at 3 min and 350 F in a convection are not properly baked, according to a baker’s experience. Cracks are another important factor which measure the ratio of crack area to land area. Deeper and wider crack patterns are preferred for this style of cookies, rather than fine and shallow crack patterns. The crack ratio in a range of 1.12 to 1.37 (result from reel oven test) was used as one of the references to justify the best baking conditions for the convection (“G”, “H”, and “I” area in Figure 17 in red) oven.

More than one response was represented in Figure 18, such as TAC, spread, and crack. The range of baking conditions which produced most acceptable cookie characteristics can then be defined (labeled area in Figure 18). Based on baking

experience, baking condition of 360 F and 4min was selected for convection oven to retain maximum TAC and desired cookie characteristic.

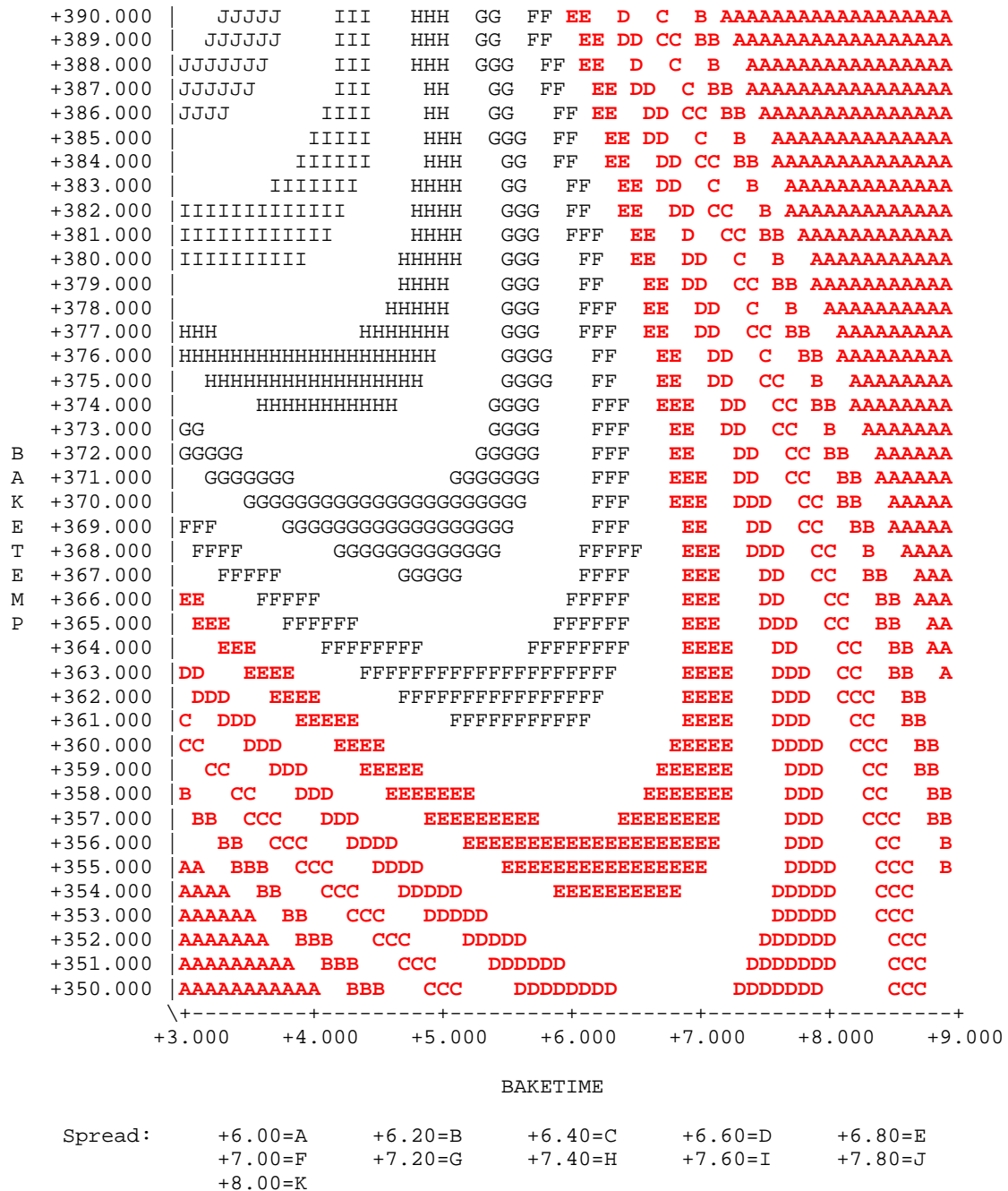


Figure 16. Effect of baking time and temperature on TAC of blue corn cookies made in air-forced convection oven



Note: BAKETIME (min), BAKETEMP (F)

Figure 17. Effect of baking time and temperature on spread of blue corn cookies made in convection oven



Note: BAKETIME (min), BAKETEMP (F)

Figure 18. Effect of baking time and temperature on cracks of blue corn cookies made in convection oven

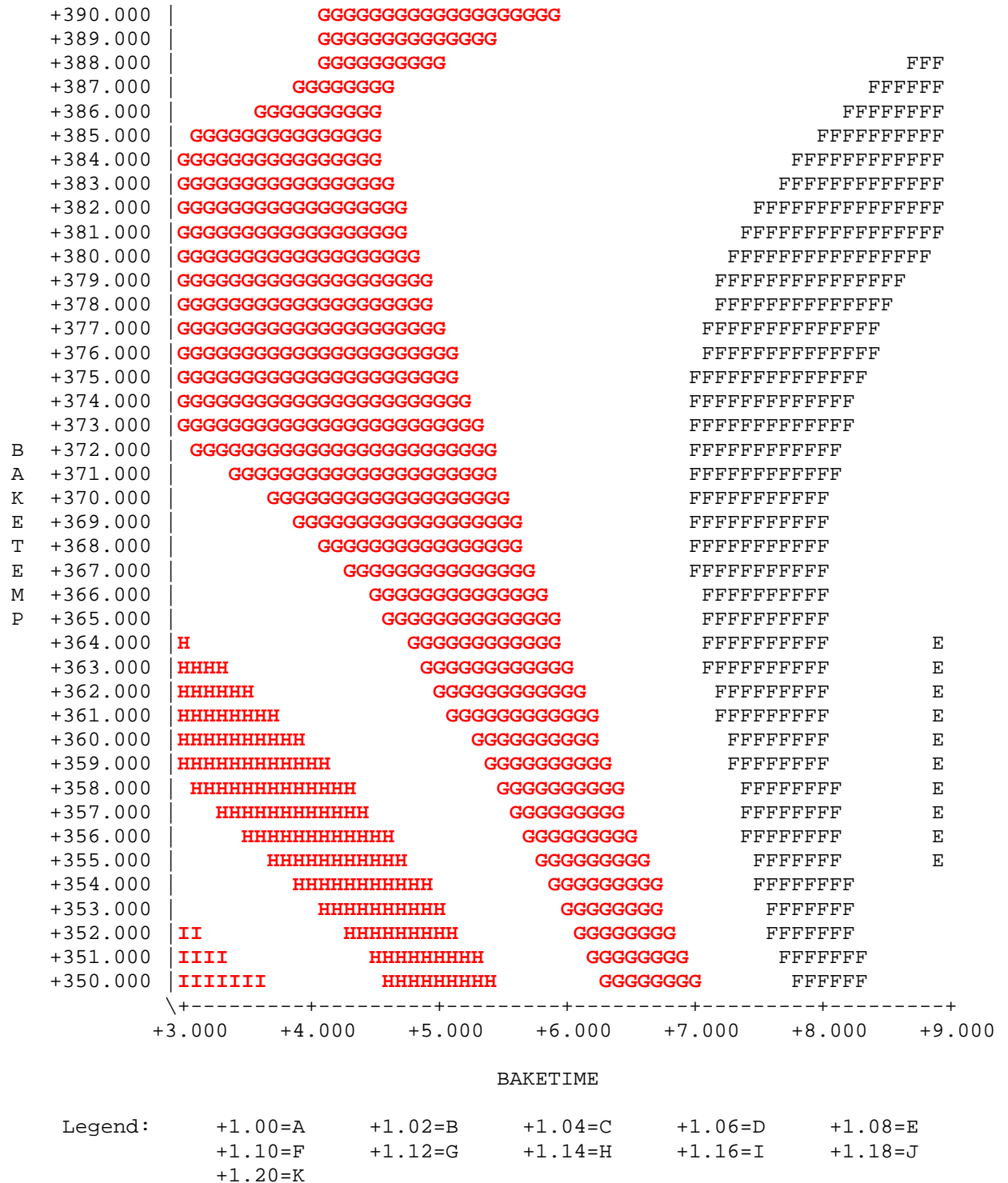
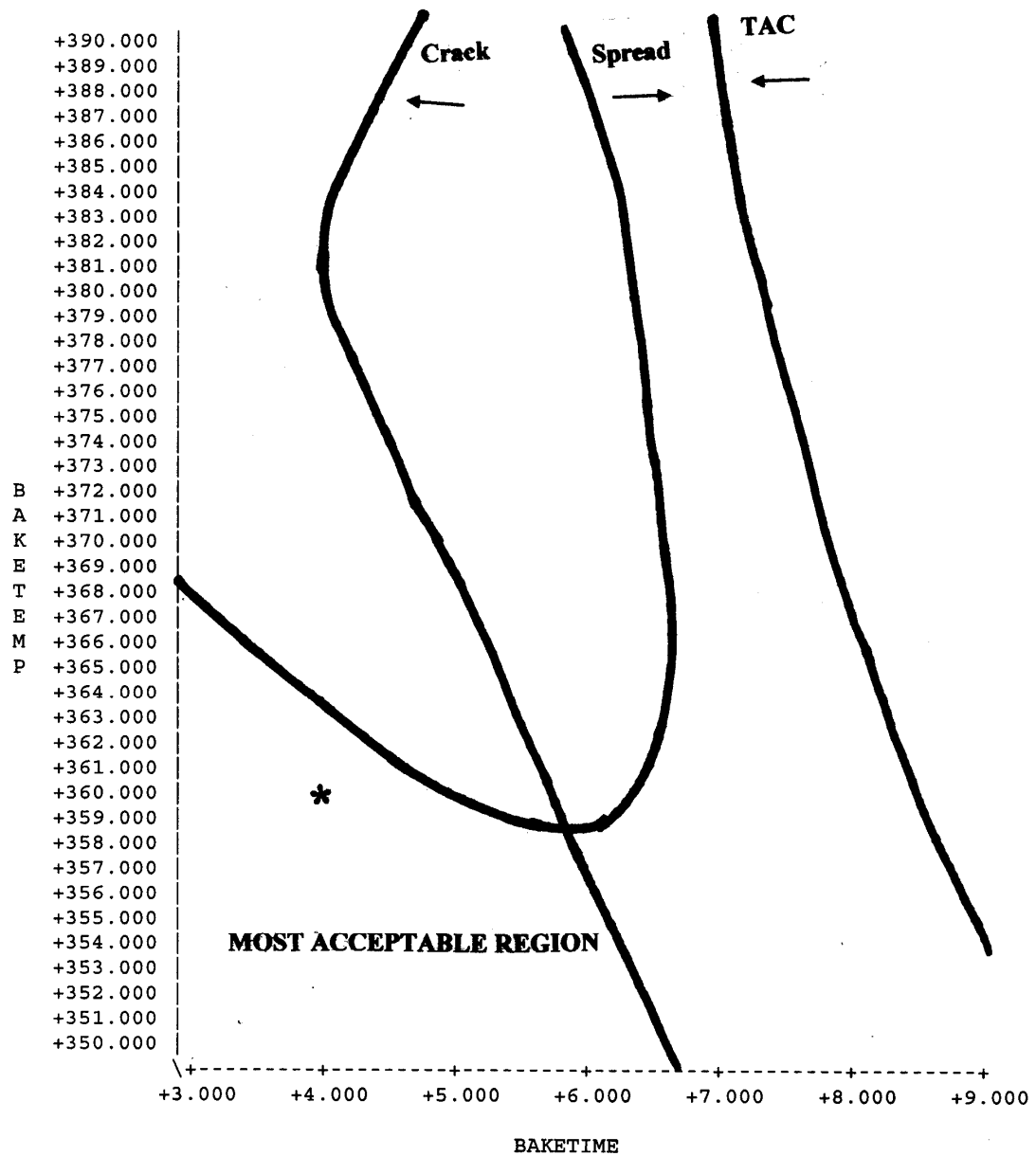


Figure 19. Most acceptable region of baking conditions for convection oven to maintain TAC



Note: Bake time (min); Bake temperature (F)

### ***Impingement oven***

The air-jet impingement oven is a form of convection oven, in which high velocity air in the form of jet is forced perpendicularly against the food product (Dogan and Walker 1999). This design was originally developed to bake pizzas, but it is equally satisfactory for other relatively thin products, such as crackers, cookies, pastries, and pies. It has the highest h-value (Table 1), which results in reduction of both baking time and oven temperature. It is generally accepted that foods baked in impingement ovens can usually be baked at about 20-25 °C lower temperature and about 50-60% shorter time as in a conventional oven; the values vary depending on the product characteristics (Li and Walker 1996). Cookies have a low moisture content and high density and normally require a relatively short baking time; therefore the reduction of baking time was reported at only about 10% in the impingement oven. And most characteristics of cookies baked in impingement oven are similar to those baked in convectional ovens (Dogan and Walker 1999).

The TAC in blue corn cookies baked in an impingement oven demonstrated a similar inverse relation with baking temperature and baking time as do the reel and convection ovens. But cookies made in the impingement oven (380 F / 7 min, “B” in bold in Figure 19) predicted higher TAC than those from reel and convection oven (132, 115, and 100 mg/kg, respectively). Blue corn cookies were baked much more efficiently in the impingement oven than in the reel oven, resulting in retaining more TAC in the cookies.

Spread demonstrated a rapid increase with oven temperature change at the short baking times. For example, at a baking time of 4 min, cookie spread was predicted to increase from 6 to 7.6 when oven temperature increased from 340 F to 380 F. When the baking time was longer than 7 min, spread became slowly reduced with an increase in baking temperature (Figure 20). This can be explained by the high efficiency of the impingement oven. From baking experience, the cookies were close to being done (the edge and bottom of the cookies appeared to be lightly brown) at 340F for 7 min. If bake time is longer or oven temperature is higher, more moisture was baked off, which

reduced the volume of the aqueous phase in the cookie dough system, thus reducing the spread (Pyler 1988).

Cracks showed a negative response to increasing baking time and temperature (Figure 21). The combination of high temperature and short time provided a higher crack ratio than the combination of low temperature and long time. This is because during baking, the structure of the center of the cookie dough usually sets up later than the dough surface because of the temperature difference in dough locations. As soon as the dough center's structure sets, the crack pattern is set. Cracks were associated with the melting rate of sugar and shortening mixture of the cookie dough during baking (Walker 1993). The higher the heat transfer rate, the deeper the crack pattern, which means the crack ratio is higher. Since heat transfer is very rapid in the impingement oven, the mixture of sugar and shortening melts faster and the surface structure sets earlier. This allows the crack pattern to be deeper at the high temperature and short time combination.

Three equations 10, 11, and 12, for TAC, Spread, and Cracks provide the relation to baking time / temperature ( $R^2 = 0.94, 0.79, \text{ and } 0.83$ , respectively). These empirical models can predict the responses (TAC, spread, and crack, respectively) from all combinations of the independent variables (baking temperature and baking time). The advantage is that it can be used to predict combinations not actually run.

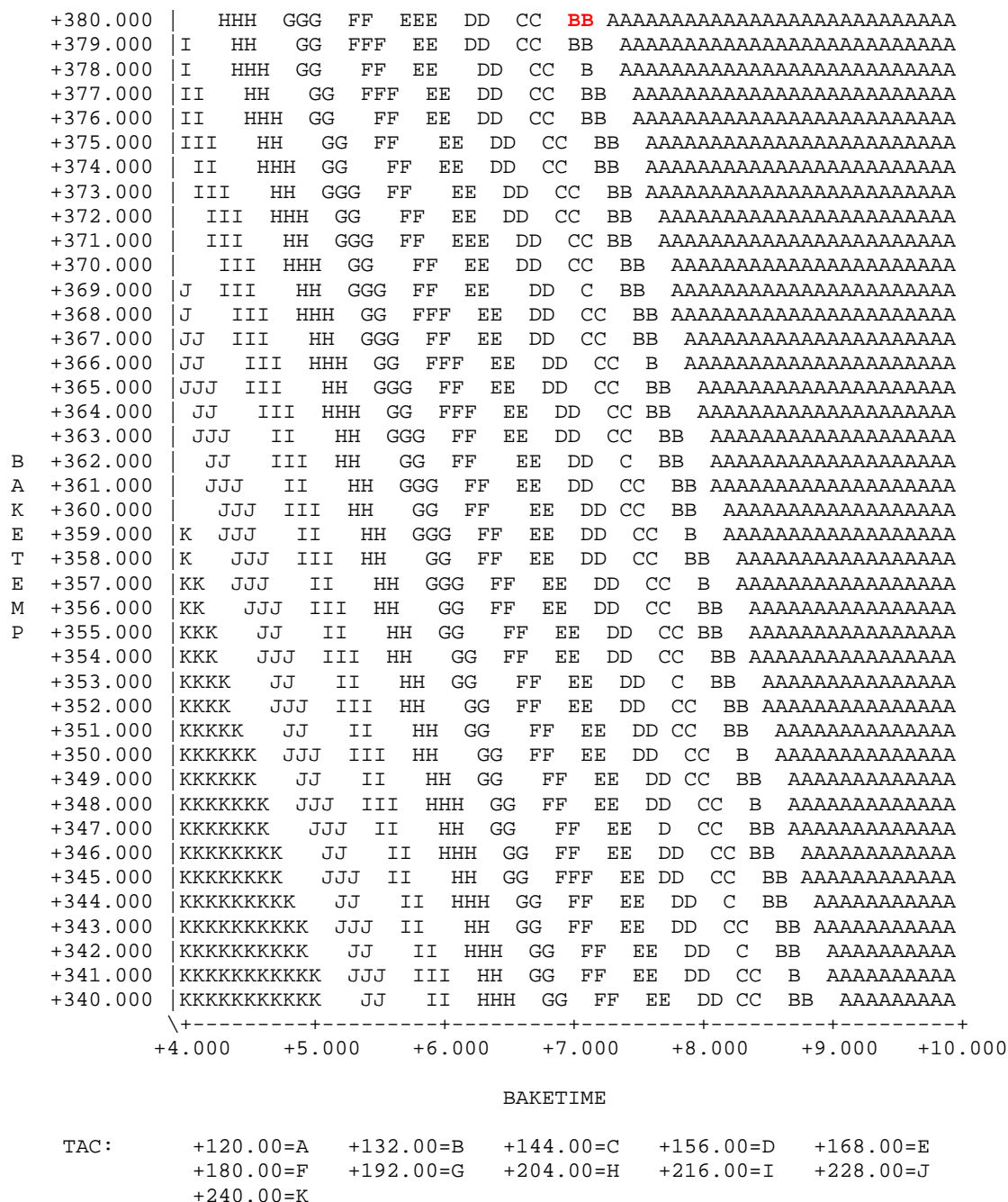
$$\begin{aligned} \text{TAC} = & 810.21 + 13.83 * \text{BAKE TIME} - 1.92 * \text{BAKE TEMPERATURE} \\ & - 0.063 * \text{BAKE TIME} * \text{BAKE TEMPERATURE} \\ & - 1.52 * (\text{BAKE TIME})^2 + 0.0014 * (\text{BAKE TEMPERATURE})^2 \end{aligned} \quad (10)$$

$$\begin{aligned} \text{SPREAD} = & -103.57 + 5.73 * \text{BAKE TIME} + 0.51 * \text{BAKE TEMPERATURE} \\ & - 0.014 * \text{BAKE TIME} * \text{BAKE TEMPERATURE} \\ & - 0.062 * (\text{BAKE TIME})^2 - 0.00055 * (\text{BAKE TEMPERATURE})^2 \end{aligned} \quad (11)$$

$$\begin{aligned} \text{CRACK} = & 2.41 - 0.19 * \text{BAKE TIME} - 0.0016 * \text{BAKE TEMPERATURE} \\ & + 0.00038 * \text{BAKE TIME} * \text{BAKE TEMPERATURE} \\ & + 0.0026 * (\text{BAKE TIME})^2 \\ & - 0.0000027 * (\text{BAKE TEMPERATURE})^2 \end{aligned} \quad (12)$$

TAC > 103 mg/kg,  $5.7 < \text{spread} < 6.9$ , and  $1.12 < \text{crack ratio} < 1.37$  was used again to select the best baking condition for impingement oven to retain maximum TAC and other desired characteristic of cookies. TAC in all combinations for the impingement oven was higher than the reference value which was obtained from the reel oven. Therefore, there is no limitation from the TAC requirement. The range of suggested baking conditions was defined, which is still a broad range (marked area in Figure 22). The baking condition could be either the combination of 355 F and 4min or the combination of 380F and 10 min. However, considering TAC for the low temperature and short time combination was higher (240 mg/kg, “K” area in Figure 19) than for the high temperature and long time combination (120 mg/kg, “A” are in Figure 19). Therefore, the low temperature and short time combination was preferred. Based on baking experience, baking conditions of 355 F and 4 min was selected for the impingement oven to retain maximum TAC and desired cookie characteristic.

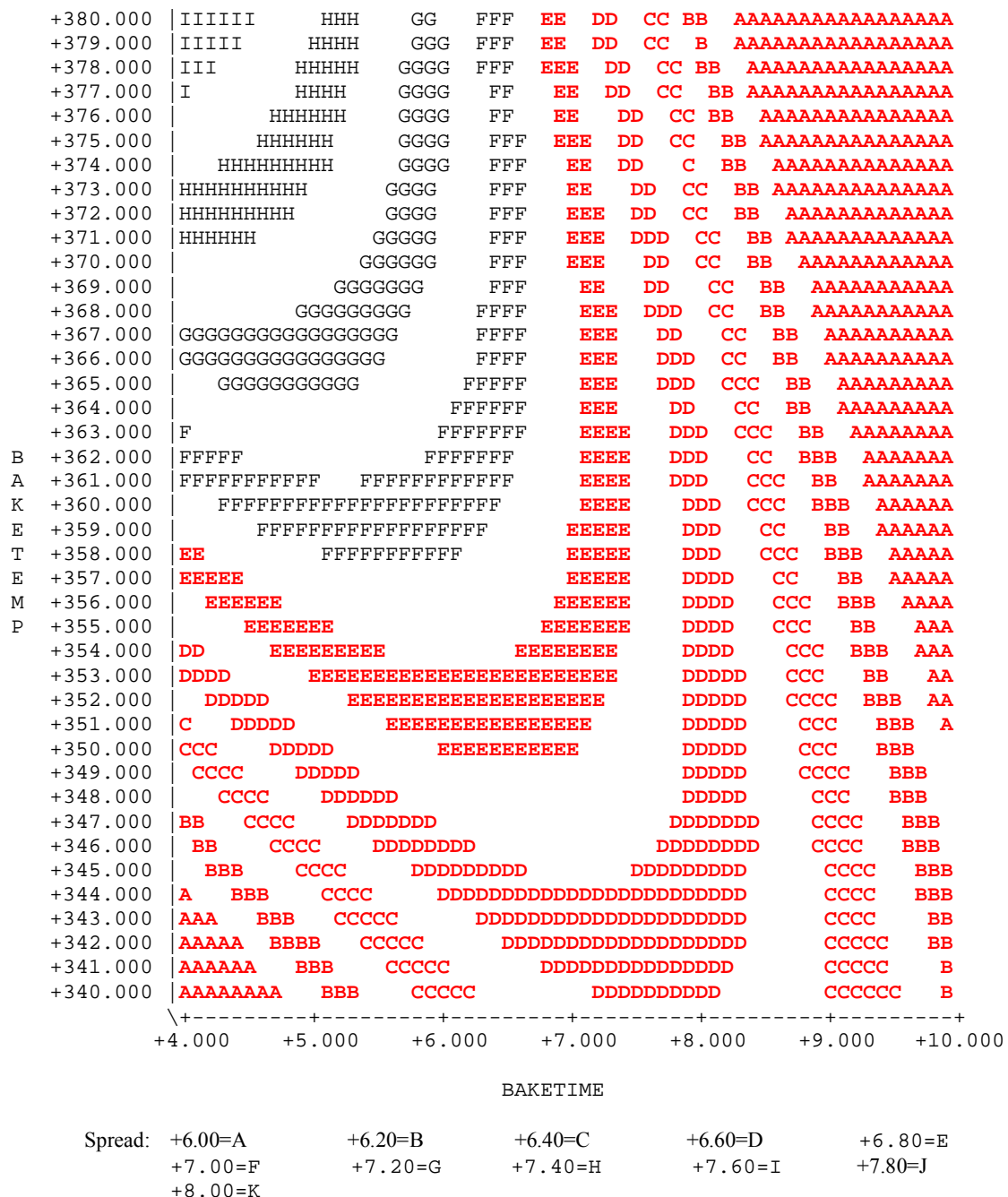
Figure 20. Effect of baking time and temperature on TAC of blue corn cookies made in an impingement oven



Note: Bake time (min); Bake temperature (F)

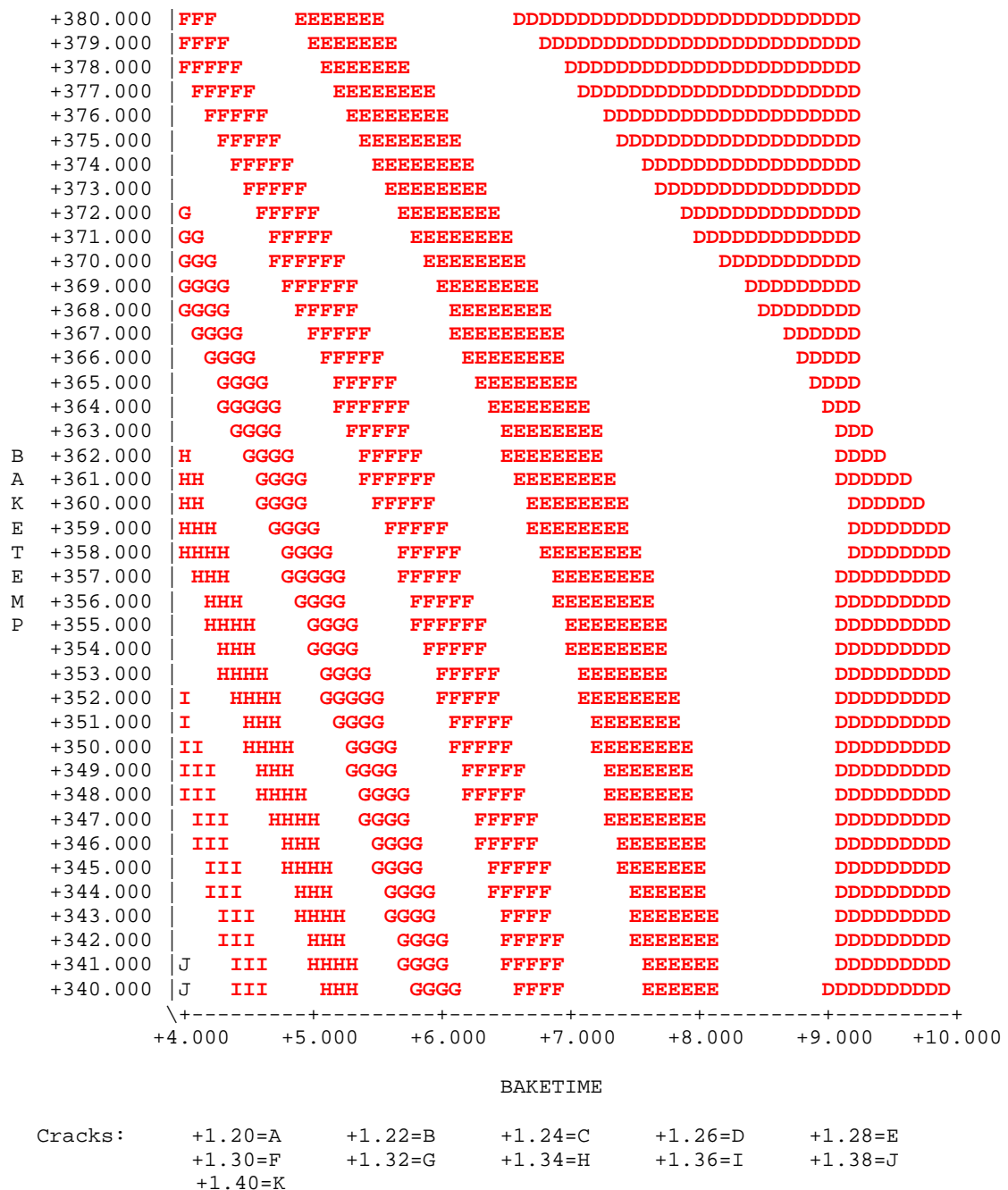


Figure 21. Effect of baking time and temperature on spread of blue corn cookies made in impingement oven



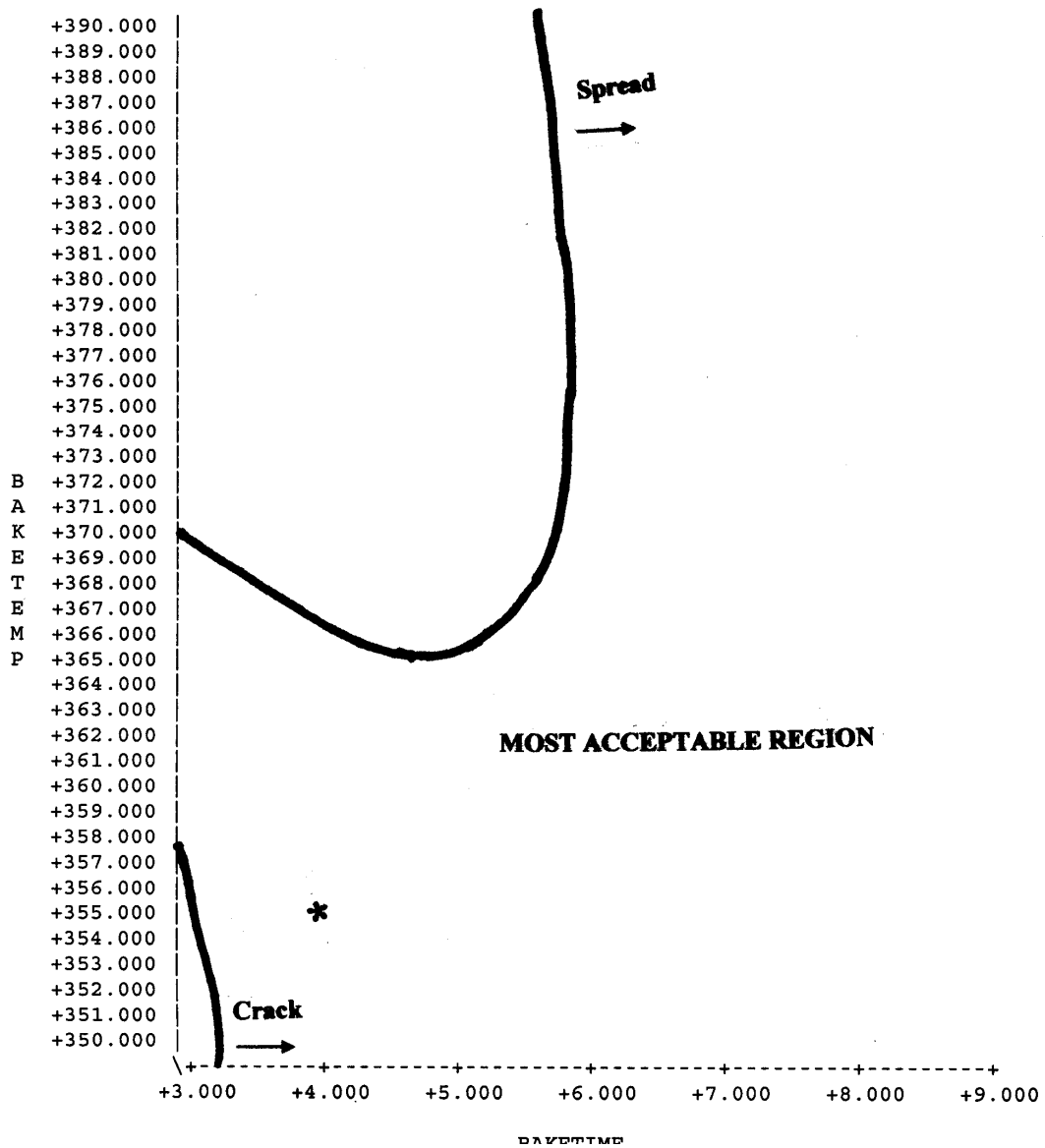
Note: Bake time (min); Bake temperature (F)

Figure 22 Effect of baking time and temperature on cracks of blue corn cookies made in impingement oven



Note: BAKETIME (min); BAKETEMP (F)

Figure 23. Most acceptable region of baking conditions for impingement oven to maintain TAC



Note: BAKETIME (min); BAKETEMP (F)

### ***Interaction of acid addition and oven type***

All the acid treatments reduced pH both in the doughs and cookies (Table 12). The citric acid treatments showed the most distinct effect followed by lactic acid and GDL, which matched the theory that their intensity of sourness is attributed by the hydrogen ( $H^+$ ) or hydronium ( $H_3O^+$ ) ion concentrations (Berry 2001). This also caused the TAC in citric acid added cookies baked in all three ovens to be higher than that of the other two acids-added cookies (Table 13).

Table 12. pH in doughs and cookies affected by interaction of acid and oven

Acid	Oven	Temperature	Time(min)	Ave. pH <sub>dough</sub>	Ave. pH <sub>cookie</sub>
lactic	impingement	179.4C (355F)	4	6.39	6.49
GDL	impingement	179.4C (355F)	4	6.89	7.18
citric	impingement	179.4C (355F)	4	5.04	5.05
lactic	reel	204.4C (400F)	10	6.40	6.57
GDL	reel	204.4C (400F)	10	6.81	7.28
citric	reel	204.4C (400F)	10	5.05	5.13
lactic	convection	182.2C (360F)	4	6.40	6.71
GDL	convection	182.2C (360F)	4	6.81	7.31
citric	convection	182.2C (360F)	4	5.05	5.15

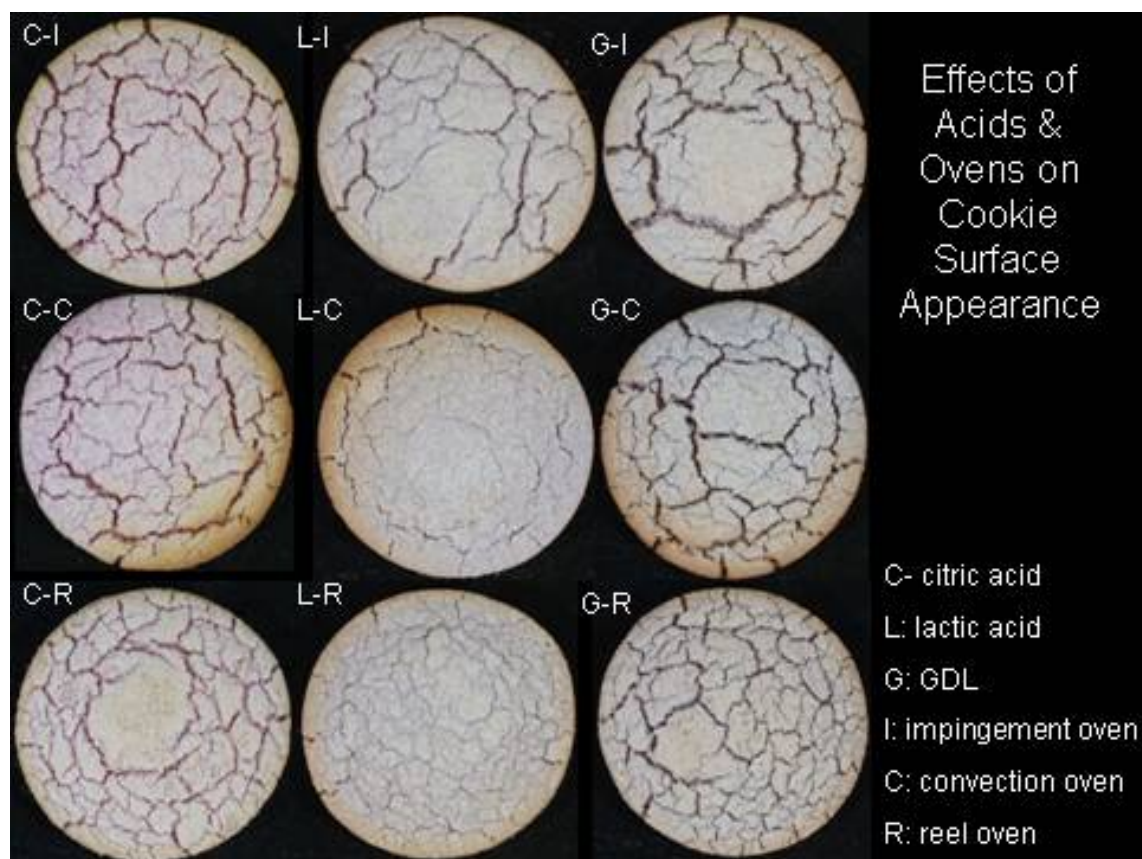
TAC in cookies was lower than that in doughs due to anthocyanins' thermal degradation. TAC in the blue corn cookies without acid ("H" area in Figure12 under the combination of 400F and 10min by reel oven) was predicted to be 103 mg/kg. It is obvious that TAC with acid supplement baked by the more efficient heat transfer ovens (impingement and convection) were much higher than TAC in the cookies without acid baked by reel oven. Adom and Liu (2002) claimed that the majority of phenolics in grains is present in the bound form (85% in corn, 75% in oats and wheat, and 62% in rice), although the free phenolics, which are at much lower levels, are more commonly reported in the literature. The combined effect of acid usage (to enhance anthocyanins' stability at lower pH) and ovens usage (more efficient in heat transfer compared to the reel oven) might release more free flavonoids including anthocyanins, which lead to an increased level of TAC in the cookies with all the combined treatments of acids and oven types (Table 13).

Figure 24 displayed the effects of combination of each acid and oven type on cookie surface appearance. The lactic acid supplements brought shallower and finer crack

Table 13. TAC, spread, texture, and moisture content (m.c.) affected by interaction of acids and ovens

Acid	Oven	Temperature	Time(min)	TAC <sub>cookie</sub> (mg/kg)	TAC <sub>dough</sub> (mg/kg)	Spread	Texture(g)	m.c. (%)
lactic	impingement	179.4C (355F)	4	210.6 ± 4.4	236.9±4.8	6.17±0.16	13859.2±633.9	5.30±0.19
GDL	impingement	179.4C (355F)	4	210.0±4.0	236.5±9.4	6.46±0.16	9465.4±41.4	5.16±0.05
citric	impingement	179.4C (355F)	4	213.5±6.8	261.1±10.7	6.22±0.05	15651.8±162.6	6.10±0.13
lactic	reel	204.4C (400F)	10	193.4±6.7	235.0±4.1	5.90±0.15	10192.0±262.5	4.87±0.21
GDL	reel	204.4C (400F)	10	183.5±5.7	243.3±5.8	6.17±0.02	7992.1±363.2	5.13±0.70
citric	reel	204.4C (400F)	10	202.5±4.1	270.5±4.9	5.92±0.16	14041.0±468.8	5.15±0.34
lactic	convection	182.2C (360F)	4	222.2±6.1	235.0±4.1	5.98±0.04	4117.9±259.0	5.75±0.87
GDL	convection	182.2C (360F)	4	220.4±1.7	243.3±5.8	6.56±0.16	8427.7±700.2	5.53±0.52
citric	convection	182.2C (360F)	4	227.7±3.4	270.5±4.9	6.71±0.10	2333.2±396.3	6.26±0.68

Figure 24. Effects of acids and ovens on cookie surface appearance



patterns in all types of oven compared to other two acid supplementations. Citric acid supplementation brought pinker color on the cookies in all three oven treatments, compared to other acid treatments, which indicated more anthocyanins remained after baking in the cookies. This trend also confirmed with the results of TAC measurement (Table 13).

According to the SAS analysis, TAC in cookie dough was mainly affected by acids rather than oven types. Treatment “citric acid” had the highest TAC level in cookie dough; but there is no significant difference between the treatment GDL and lactic acid. However, TAC in the final cookies was affected by both acid and oven types. Citric acid still demonstrated the highest TAC level. The treatments GDL and lactic acid didn’t show significant difference. The cookies baked in the convection oven contained the highest TAC followed by the impingement oven and then reel oven, due to relatively higher heat transfer coefficients (h-value) compared to natural convection oven (reel oven). The impingement oven bakes faster than in conventional baking (up to 50% shorter in time), especially in thin layer products like pizza. But it is limited by the internal thermal transfer characteristics of the foods. It takes time for the heat to penetrate to the interior for thick products (Walker and Xue 2008). This is why cookies baked in impingement ovens didn’t gain bigger advantage over the cookies baked in convection oven, although the h-value of impingement oven is higher than that of convection.

Cookie spread (AACC method) is affected by both acid and oven types. The treatment with GDL generated the largest spread, then followed by citric acid, then lactic acid. The cookies baked by either convection or impingement ovens generated higher spreads than those from the reel oven.

Table 14 lists the cookies’ diameter, spread, eccentricity, brightness, and crack ratio generated from Surfscan image-analyzer program (AEW Consulting, Lincoln, NE). GLM of SAS analysis indicated that it is type of acids (lactic, GDL, or citric acid) rather than oven types (impingement, reel, or convection oven) affect blue corn cookies’ quality indices.

Citric acid is the strongest acid among the three organic acids, which dramatically lowered the pH in the doughs and cookies, thus increased TAC retention. However, its effect on diameter, and crack ratio is second to the treatment by GDL, and similar in

function to eccentricity as was GDL. The effect of treatment GDL is weakest in terms of the brightness of cookies.

Crack ratio is defined by the total dark area to the total area of the cookies, separating the dark areas of the image (pixels) from the light area (Dogan and Walker 1999). The crack ratio from GDL and citric acid treatments are higher than that from lactic acid treatment. This means the cookies with lactic acid treatment displayed a finer and shallower crack pattern.

Table 14. SurfScan analysis

ACIDS	OVENS	DIAMETER INCHES	SPREAD % CUTTER	ECCEN. % AREA	BRIGHTNESS RAW	CRACKS RATIO
lactic	imp	2.75	16.37	2.60	153.20	1.17
lactic	reel	2.76	17.02	2.64	150.08	1.15
lactic	conv	2.74	15.84	2.47	154.97	1.12
GDL	imp	2.97	25.68	3.29	132.02	1.31
GDL	reel	2.98	26.02	3.55	134.00	1.26
GDL	conv	2.94	24.59	3.49	144.60	1.28
citirc	imp	2.88	21.90	2.91	154.03	1.27
citric	reel	2.90	22.68	3.34	143.70	1.18
citric	conv	2.91	23.23	2.69	158.08	1.20

Note: imp = impingement oven; reel = reel oven; conv = convection oven

## Summary of Results

1. GDL, citric acid, and lactic acid all increase TAC in both the cookie dough and in the final cookies by lowering their pH. Citric acid, with three replaceable hydrogen ions, retains the highest level of TAC in the cookies.
2. Cookies made with either GDL or citric acid generated larger spread, diameter, area, eccentricity, and crack ratio as compared to the lactic acid treatment.
3. Convection, impingement, and reel ovens significantly affected TAC in the final cookies. The cookies baked in convection ovens retained the highest levels of TAC.

4. Oven types affect cookie spread, but not eccentricity, brightness, and crack ratio.
5. The blue corn cookies made with the combination of citric acid and convection oven is considered the best in terms of maximum TAC retention; the blue corn cookies made with the combination of GDL and convection oven is considered the best in terms of cookie spread, crack, eccentricity, and other quality indices.

## **Conclusions**

Varying oven types and acidulant agents increased retention of anthocyanin in blue corn cookie. Cookie served as an example of baked food system that demonstrates the effects of manipulating ingredients and process conditions can improve anthocyanin retention.



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## Appendix A - RSM figures

Figure A.1 Response surface for TAC in blue corn cookie as affected by guar gum% and blue corn flour% (Stepping variable water% = 21.5%)  $R^2 = 0.99$

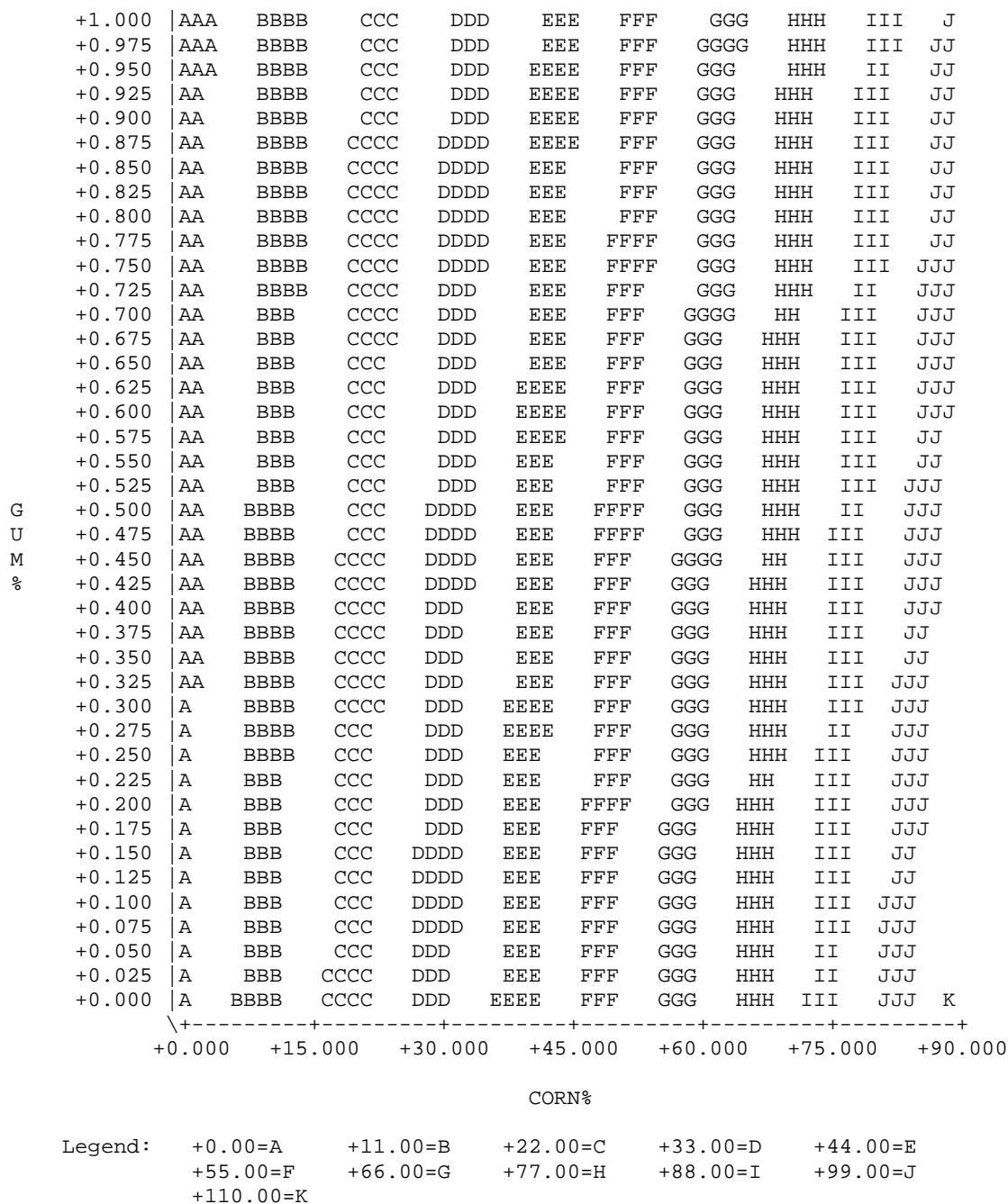


Figure A.2 Response surface for TAC in blue corn cookie as affected by guar gum% and blue corn flour% (Stepping variable water% = 24.5%)  $R^2 = 0.99$

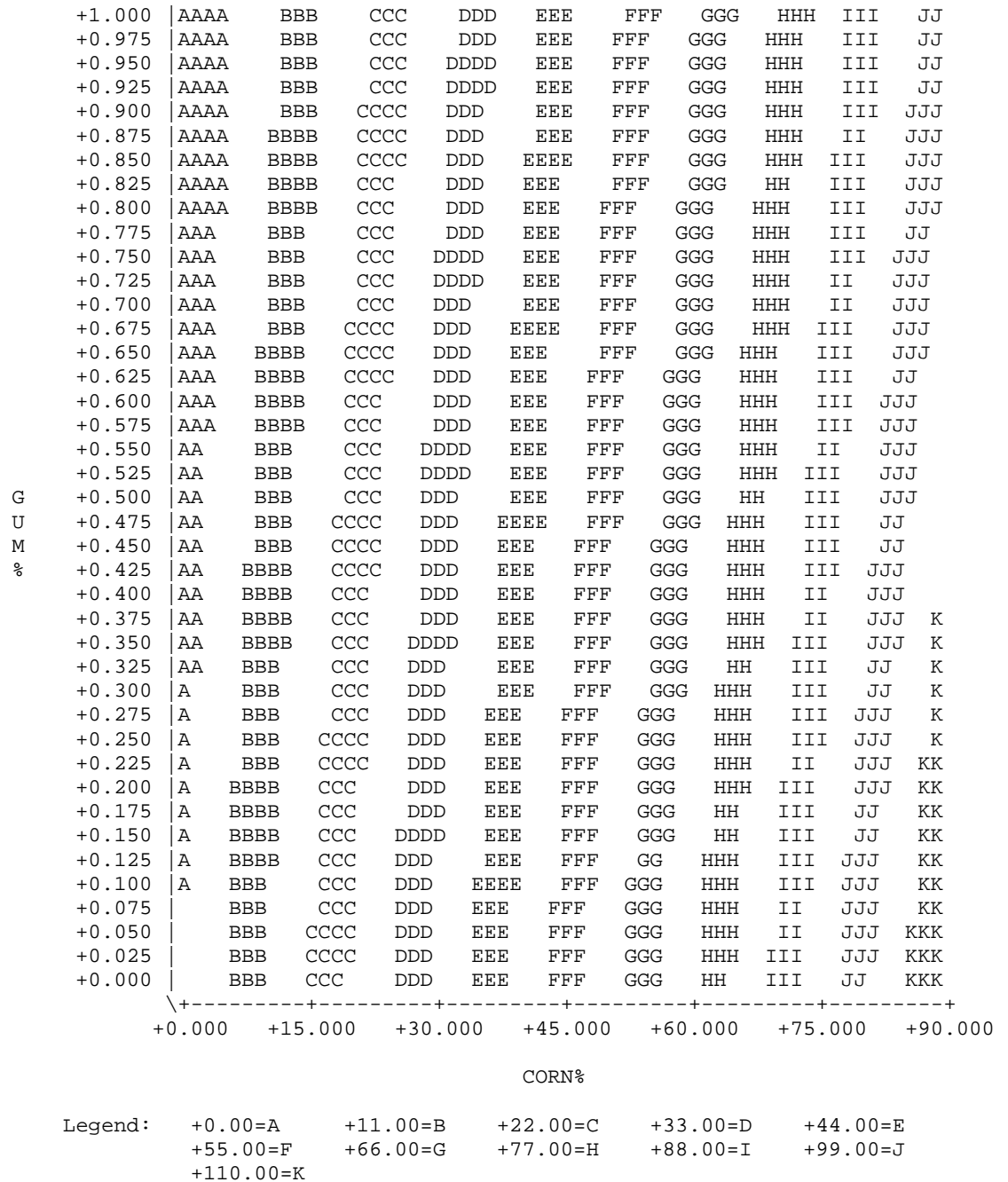


Figure A.3 Response surface for TDF in blue corn cookie as affected by guar gum% and blue corn flour% (Stepping variable water% = 21.5%)  $R^2 = 0.63$

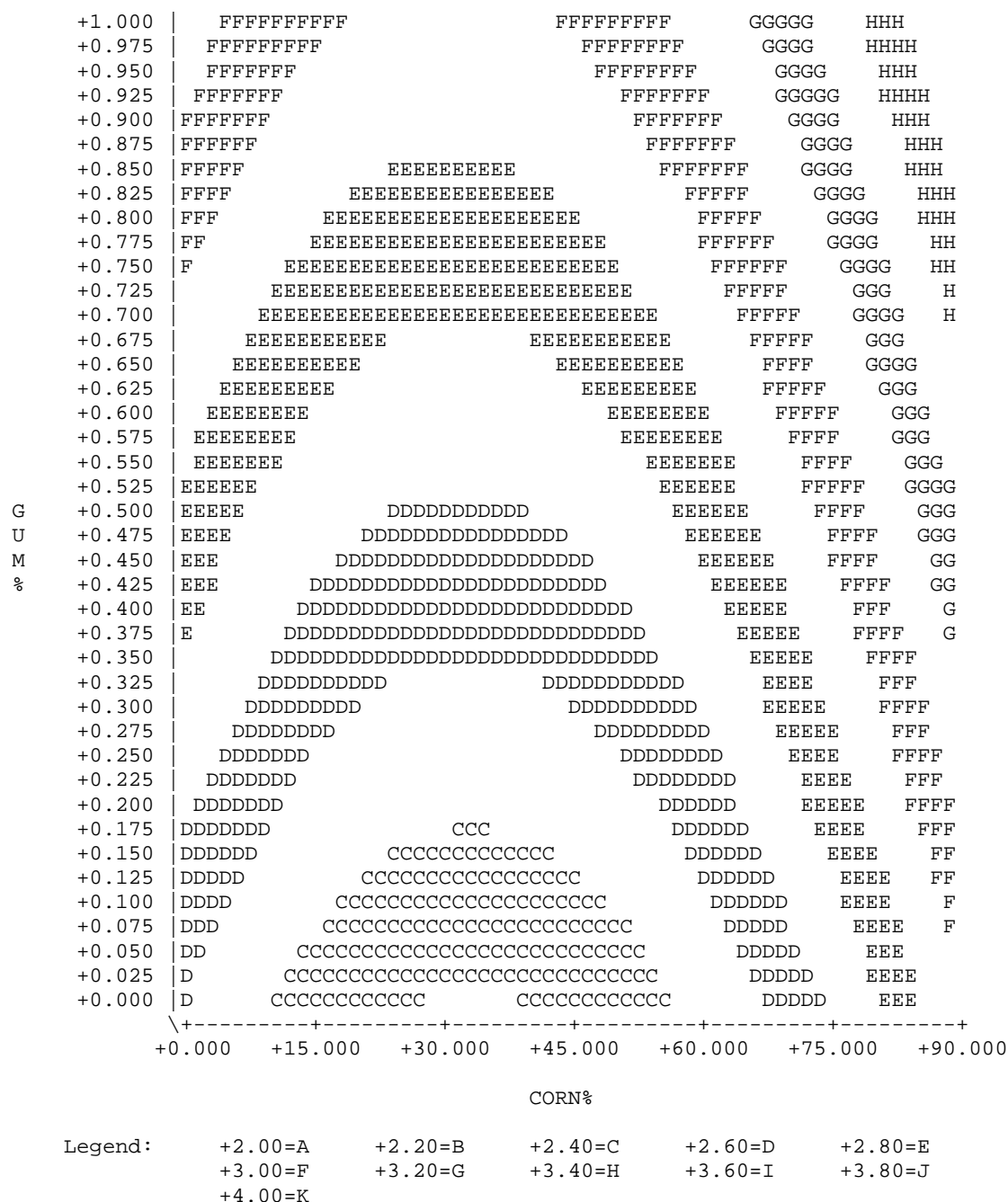


Figure A.4 Response surface for TDF in blue corn cookie as affected by guar gum% and blue corn flour% (Stepping variable water% = 24.5%)  $R^2 = 0.63$

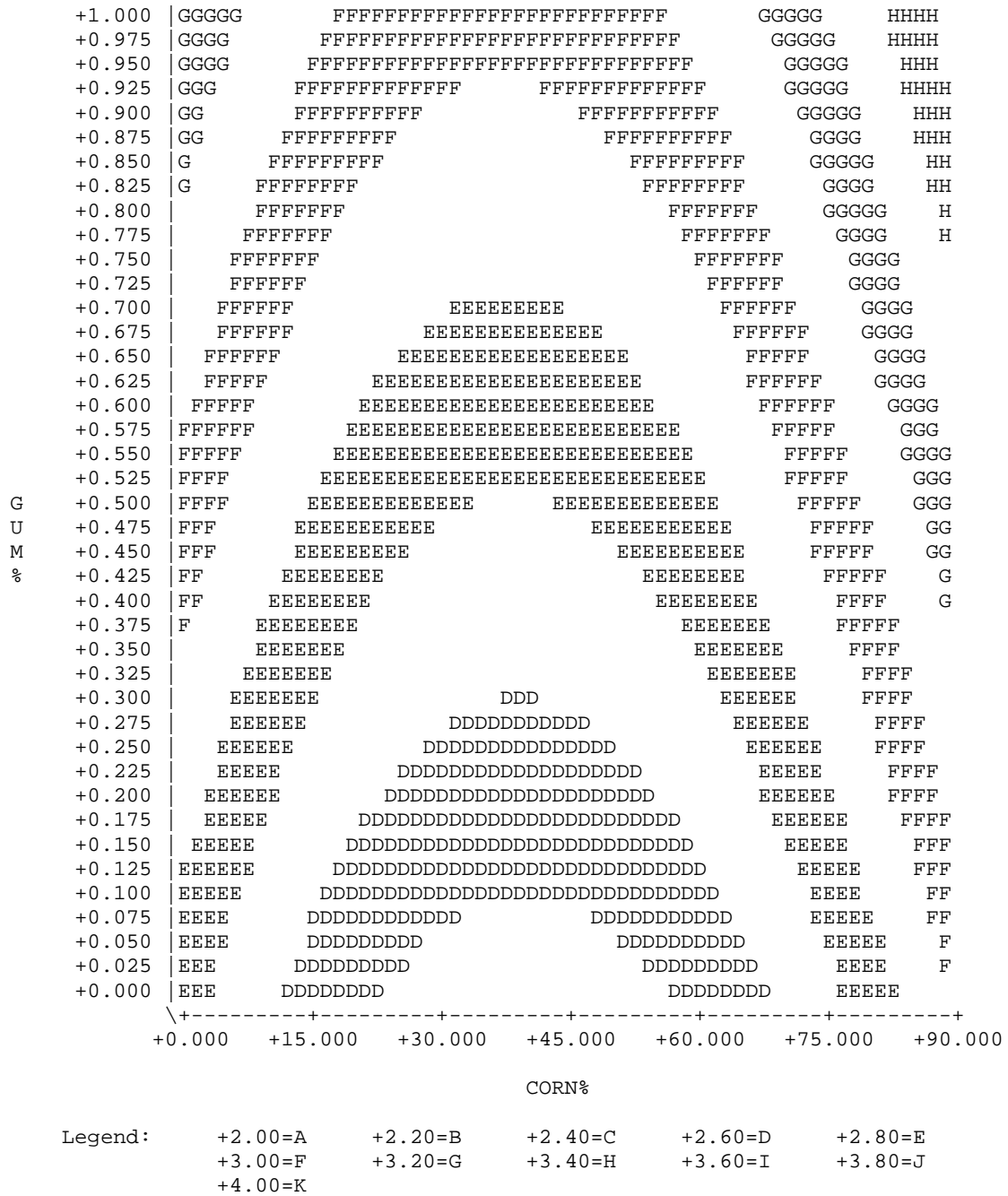


Figure A.5 Response surface for spread in blue corn cookie as affected by guar gum% and blue corn flour% (Stepping variable water% = 21.5%)  $R^2 = 0.86$

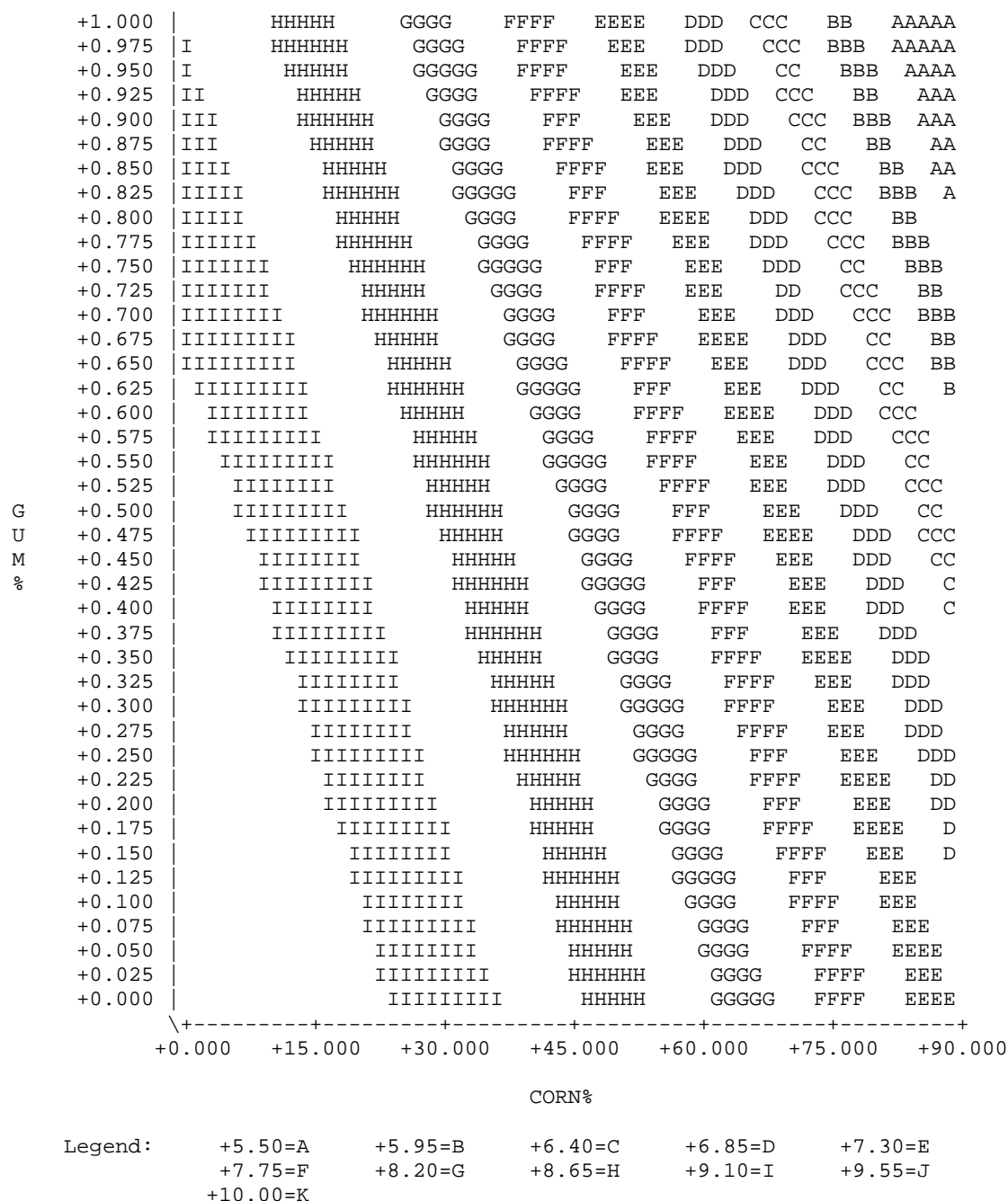




Figure A.6 Response surface for spread in blue corn cookie as affected by guar gum% and blue corn flour% (Stepping variable water% = 24.5%)  $R^2 = 0.86$

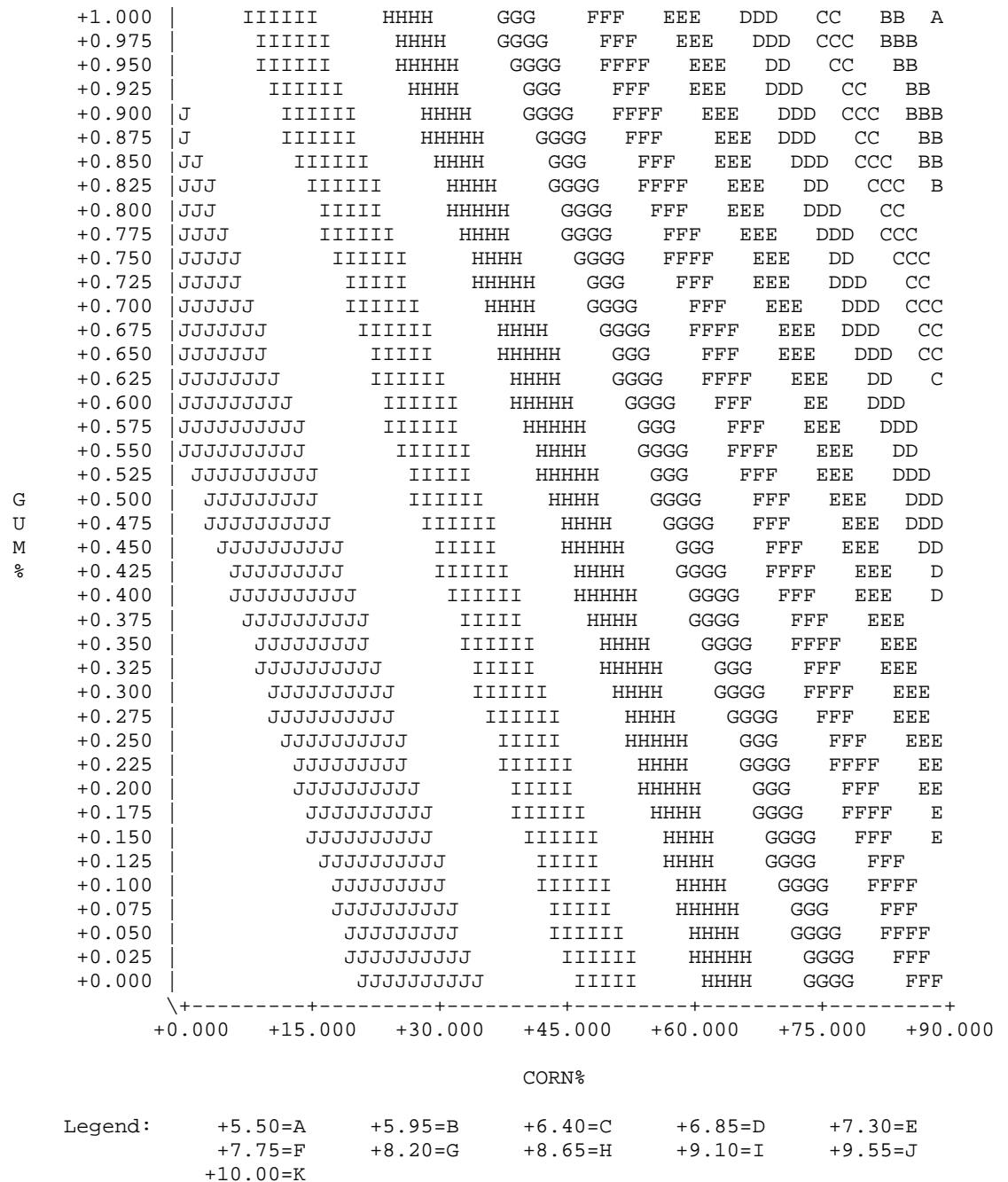


Figure A.7 Response surface for blue corn cookie texture as affected by guar gum% and blue corn flour% (Stepping variable water% = 21.5%)  $R^2 = 0.87$

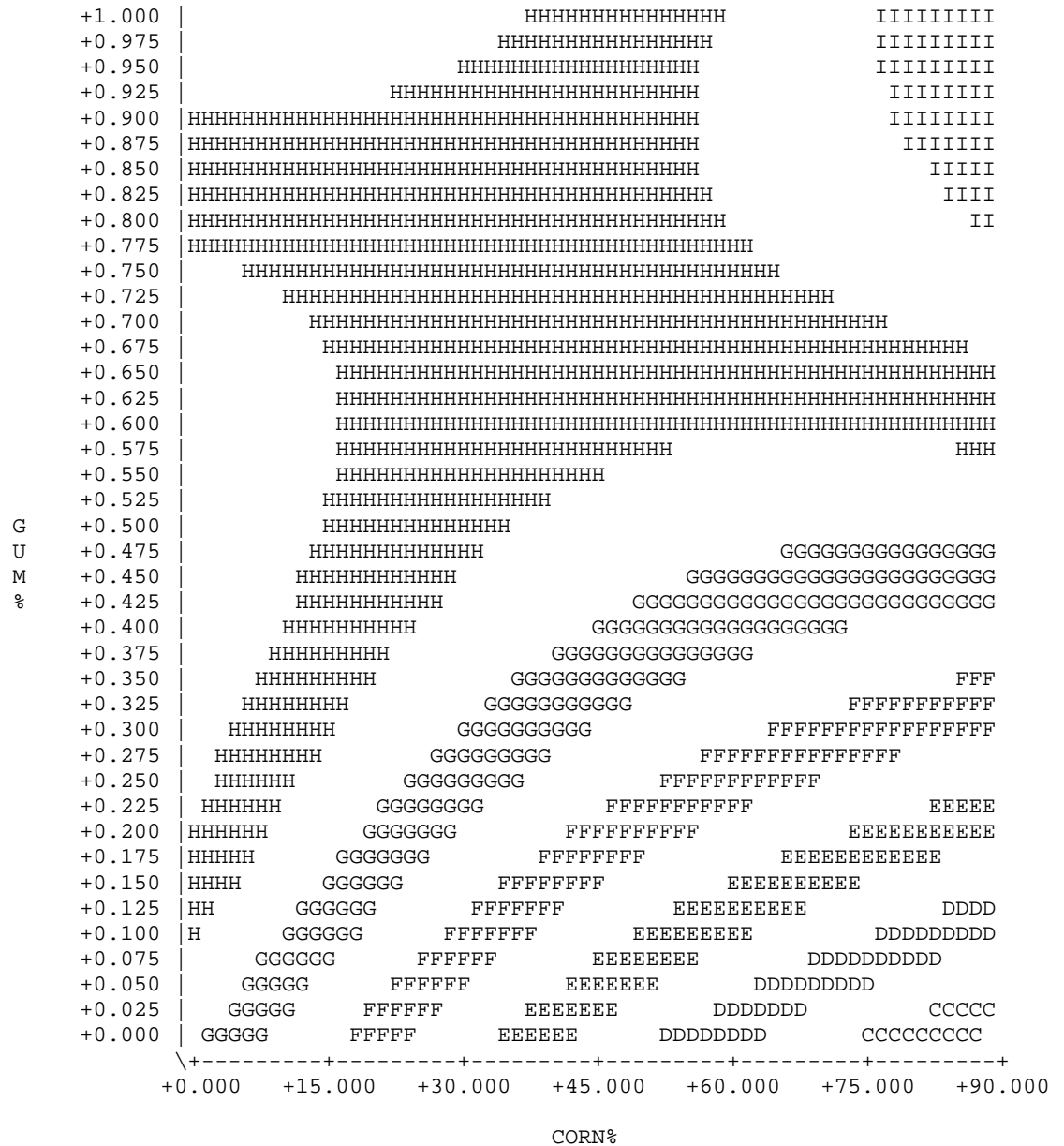


Figure A.8 Response surface for blue corn cookie texture as affected by guar gum% and blue corn flour% (Stepping variable water% = 24.5%)  $R^2 = 0.87$

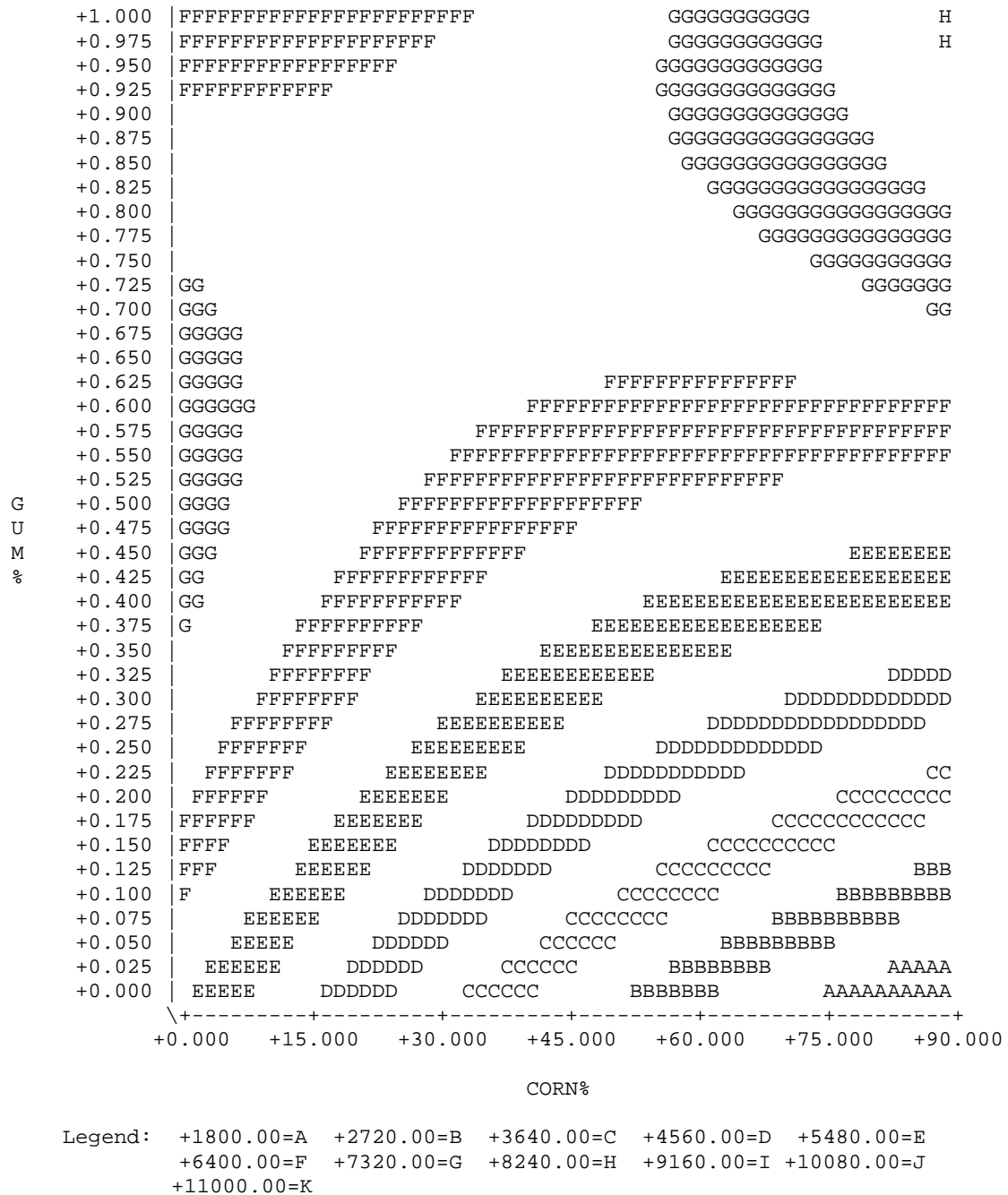


Figure A.9 Response surface for blue corn cookie brightness as affected by guar gum% and blue corn flour% (Stepping variable water% = 18.5%)  $R^2 = 0.98$

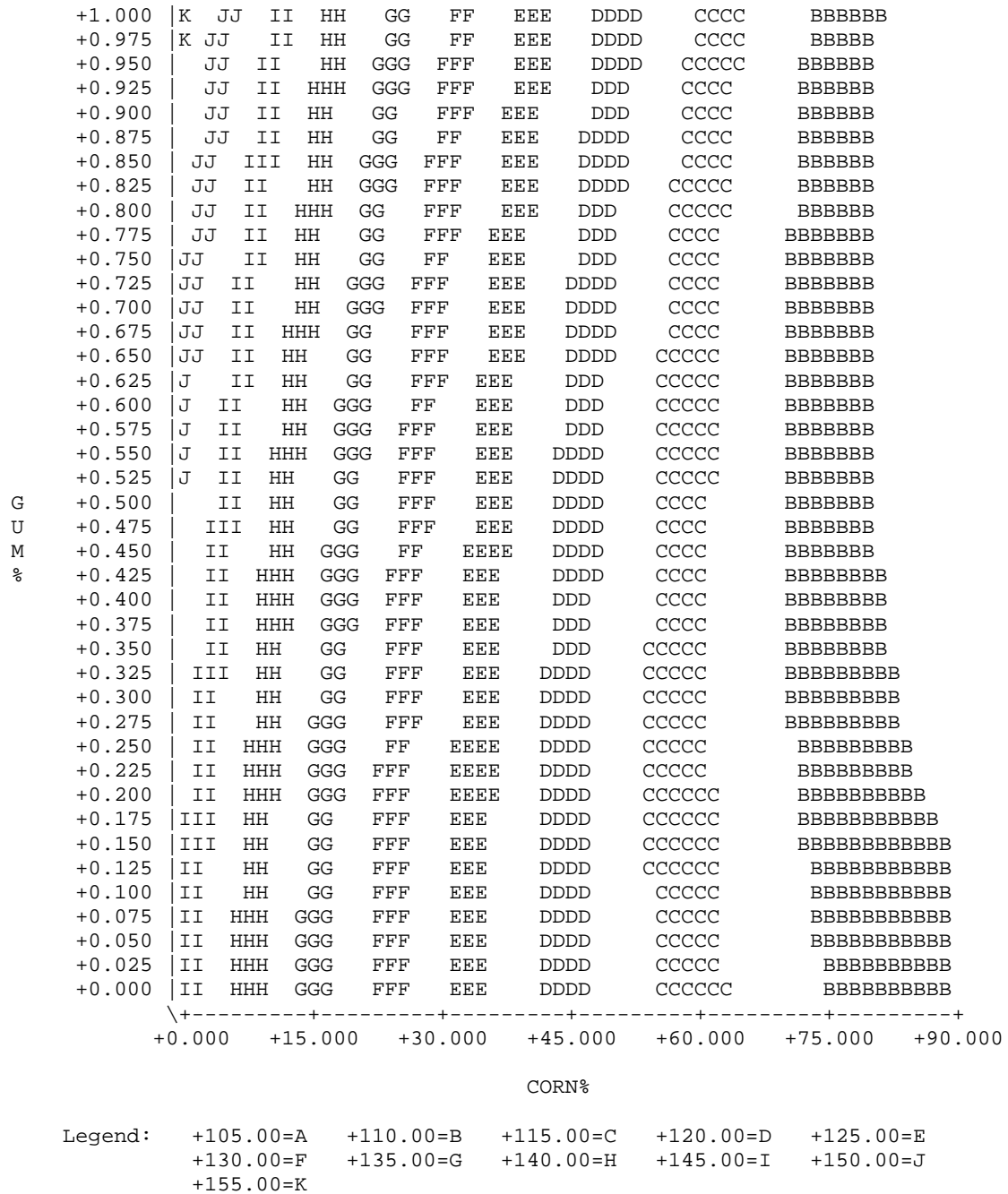


Figure A.10 Response surface for blue corn cookie brightness as affected by guar gum% and blue corn flour% (Stepping variable water% = 24.5%)  $R^2 = 0.98$

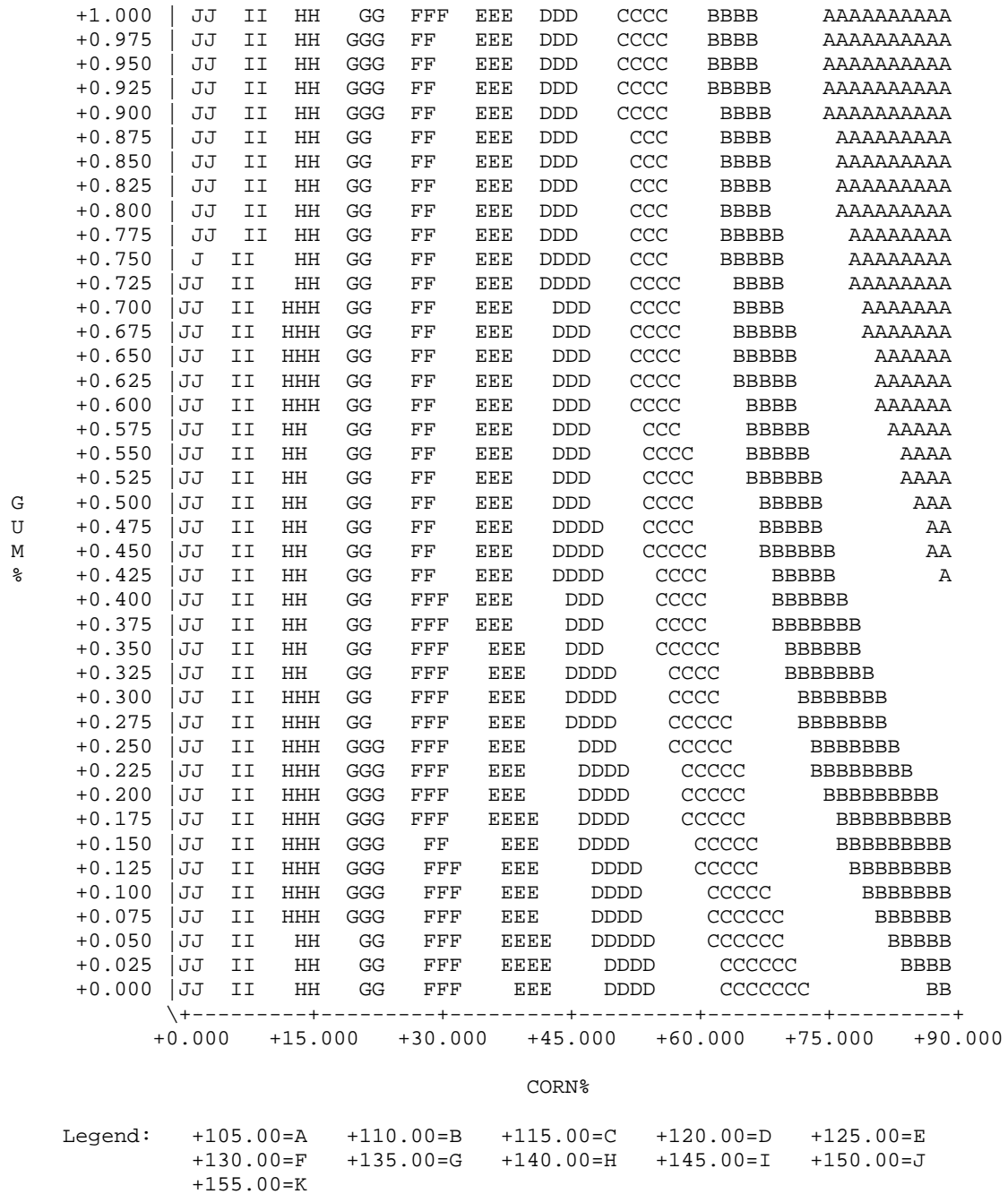


Figure A.11 Crack – reel oven

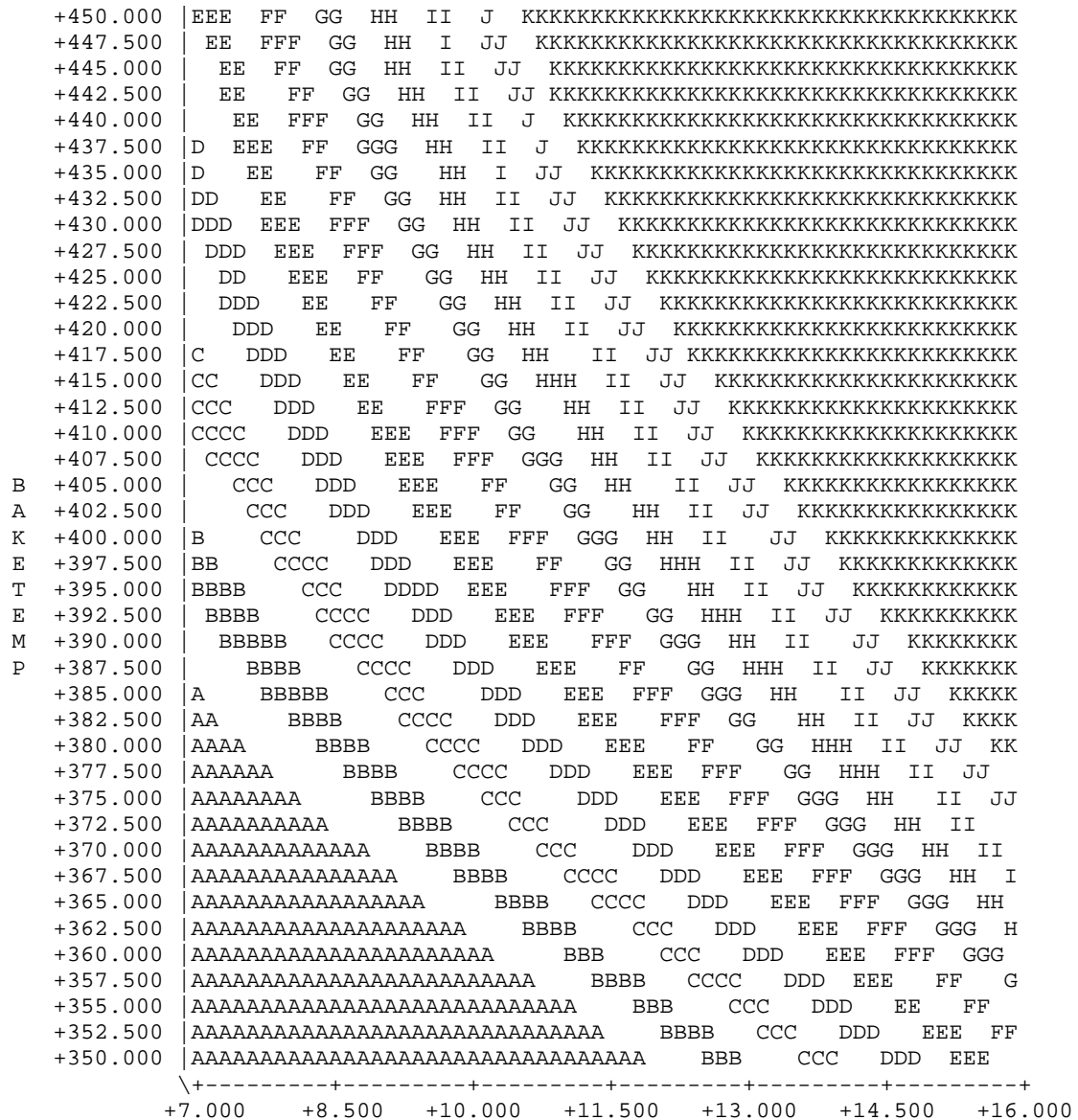


Figure A.12 Brightness-reel oven

